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Comprehensive Invited Review

SUMO Proteases: Redox Regulation and Biological Consequences

Zheng Xu, Ho Yin Chan, Wai Ling Lam, Kwok Ho Lam, Levina Suk Mi Lam, Tzi Bun Ng, and Shannon Wing Ngor Au

Reviewing Editors: Wulf Droege, Mark Hannink, David Sheehan, Thomas Squier, and Jun Wang

Centre for Protein Science and Crystallography, Department of Biochemistry and Molecular Biotechnology Program, Faculty of Science, The Chinese University of Hong Kong, Hong Kong.

Abstract

Small-ubiquitin modifier (SUMO) has emerged as a novel modification system that governs the activities of a wide spectrum of protein substrates. SUMO-specific proteases (SENP) are of particular interest, as they are responsible for both the maturation of SUMO precursors and for their deconjugation. The interruption of SENPs has been implicated in embryonic defects and carcinoma cells, indicating that a proper balance of SUMO conjugation and deconjugation is crucial. Recent advances in molecular and cellular biology have highlighted the distinct subcellular localization, and endopeptidase and isopeptidase activities of SENPs, suggesting that they are nonredundant. A better understanding of the molecular basis of SUMO recognition and hydrolytic cleavage has been obtained from the crystal structures of SENP–substrate complexes. While a number of proteomic studies have shown an upregulation of sumoylation, attention is now increasingly being directed towards the regulatory mechanism of sumoylation, in particular the oxidative effect. Findings on the oxidation-induced intermolecular disulfide of E1-E2 ligases and SENP1/2 have improved our understanding of the mechanism by which modification is switched up or down. More intriguingly, a growing body of evidence suggests that sumoylation cross-talks with other modifications, and that the upstream and downstream signaling pathway is co-regulated by more than one modifier. *Antioxid. Redox Signal.* 11, 1453–1484.

I. Introduction

Diverse functions and intricate regulatory mechanisms in eukaryotes, conferred by a limited number of genes in the genome, are mediated by a protein–protein interaction network, alteration of intracellular protein localization, and protein post-translational modifications. Post-translational modifications of proteins are key players responsible for most of the underlying mechanisms involved in protein–protein/ligand interaction and protein trafficking (55, 229, 246, 326). Modification usually involves conjugation of a protein target with a modifier that acts as a signal molecule and induces functional and/or structural changes. The consequences of protein modification by phosphorylation, acetylation, methylation, glycosylation, neddylation, and ubiquitination are closely associated with signal transduction, transcription, cell cycle control, DNA repair, and genome stability (19, 62, 109, 190, 207, 222, 283, 305).

In 1995, a 101-amino acid residue modifier, designated as SMT3 (suppressor of mif two, 3), was identified in *Saccharomyces cerevisiae* (185) and shown to be essential for the maintenance of chromosome integrity during mitosis. The name SUMO (small ubiquitin-related modifier) was later given and widely applied due to its structural similarity with ubiquitin. Nowadays, SUMO modification has emerged as a novel and indispensable protein modification system modulating the functions of a broad spectrum of proteins involved in gene expression regulation, signal transduction, and chromosome integrity (52, 82, 93, 102, 195). An increasing number of proteins have been shown to be SUMO-modified, many of which are tumor suppressor proteins, transcription factors, and nuclear body proteins. Sumoylation is clearly an important process, and a better understanding of its nature might well provide important insights into the molecular mechanisms of cancer development and therapy. Similar to other post-translational modifications, sumoylation is a dynamic reversible process consisting of SUMO conjugation and deconjugation. Apparently, the addition of SUMO moieties to, or their removal from target substrates causes immediate cellular responses to various intracellular signals and extracellular stimuli. However, apart from other modifications, there are three SUMO isoforms found in higher eukaryotes. Though SUMO-1 and SUMO-2/-3 share 50% sequence identity, they have unique intracellular abundance and specific conjugation substrates. SUMO-2/-3, like ubiquitin, can polymerize to form polySUMO chains. The presence of three SUMO E3 ligase families and six SUMO-specific proteases (SENP) found in humans today implies that the substrate modifications are specific and tightly controlled. Intriguingly, the catalytic properties of SENPs are not only restricted to the deconjugation process but are also responsible for regulating the availability of 'free' SUMOs for conjugation and depolymerization of SUMO-2/-3. Recent advances in the structure-function studies of SENPs have revealed that these enzymes possess distinct features and biological significance in cells.

II. SUMO Metabolism

SUMO protein exerts their effect by covalent attachment at specific lysine residue(s) in their target proteins. It is highly conserved in all eukaryotes; however, only a single SUMO gene is found in lower eukaryotes such as nematodes and yeasts while three SUMO isoforms are found in higher eukaryotes; they are SUMO-1 (Smt3c, PIC1, GMP1, Sentrin, and Ubl1 in humans), SUMO-2 (Smt3a and Sentrin3), and SUMO-3 (Smt3b and Sentrin2) (102). Like ubiquitin, SUMO is expressed in a precursor form that has to be processed by SUMO-specific proteases to cleave the C-terminal isopeptide bond to expose the di-glycine residues for conjugation. The structure of SUMO proteins (14, 116) is such that the N- and C-terminal regions of all mature SUMO paralogues are surface exposed and flexible. The C-terminal extension contains the GG motif for conjugation, while a consensus sumoylation site at the N-terminal region of SUMO-2/3 and SMT3 corresponds to the formation of SUMO polymer on their substrates (32, 86, 272, 276). In humans, SUMO-1 exhibits 44% sequence identity with SUMO-2 and SUMO-3, while SUMO-2 and SUMO-3 share 86% and 97% sequence identity before and after processing, respectively (Fig. 1). Recently, a fourth SUMO member, intronless SUMO-4, has been reported to be a non-expressed pseudogene (267). This polymorphic variant, harbouring a 55 Met → Val substitution of SUMO-3, is expressed in kidney cells (27). However, it is incapable of forming a covalent conjugate (209), as native SUMO-4 cannot be processed.

A. SUMO-1

SUMO-1 is the primitive isoform discovered in mammalian cells that demonstrates only 18% sequence homology to ubiquitin (239). It is localized in the nucleus, ubiquitously in many different types of cells, and generally remains in the conjugated form (240). The most abundant and well characterized of the SUMO-1 conjugated substrates known in vertebrates is Ran GTPase activating protein (RanGAP1). Using COS-7, HeLa, SK-N-M, and NB4, mouse NIH3T3 cells, and *Xenopus* egg extracts, Saitoh and Hinchey (240) showed that RanGAP1 was preferentially modified by SUMO-1, instead of by SUMO-2 or SUMO-3. The functional role of SUMO-1 modification has been investigated by using antisense oligo against *Xenopus* SUMO-1 (XSUMO-1) (315). The down-regulation of XSUMO-1 inhibits normal axis formation in embryos and elongation of activin-treated animal caps, suggesting that SUMO-1 conjugation is directly associated with activin/nodal signaling.

B. SUMO-2 and SUMO-3

These two SUMO isoforms, first isolated from human and mouse cDNA libraries, are expressed in a wide range of tissues in humans (38, 152). A sequence homology search reveals that these two isoforms are widely distributed among vertebrates, and a higher homology to SUMO-2/3 than to SUMO-1 has been detected in the insect genera *Drosophila* and *Bombyx*. However, little is known about their function when compared with their mammalian homologs (240). Although SUMO-3 is found to be a poor endopeptidase substrate, it is deconjugated as efficiently as SUMO-2 by all SENPs *in vitro* (186). A recent proteomic study has shown that the three SUMO isoforms form mixed chains *in vivo* via the sumoylation site in SUMO-2/3, while *in vitro* mixing of the relative amounts of SUMO-1 and SUMO-2 can modify the length of the poly-SUMO chain (179). For ubiquitin, the biological activity of polyubiquitination is closely related to the length and precise linkage of polyubiquitin. Although the function of a single SUMO moiety and that of polySUMO moieties are not well understood, given that SUMO can regulate the mode of protein–protein/-DNA interaction, it is possible that different numbers of SUMO attached would direct specific biological effects.

A higher intracellular concentration of "free" SUMO-2/3 observed *in vivo* suggests a potentially larger capacity of SUMO-2/3 modification than SUMO-1 modification. This is further demonstrated by the upregulation of SUMO-2/3 conjugation induced upon heat shock and oxidative stress (81, 95, 163, 173, 324). Using COS-7 cells, a rapid response to acute hyperthermia was associated with a diminished quantity of free SUMO-2/3 and an accumulation of SUMO-2/3 conjugates. By contrast, no dramatic change of SUMO-1 conjugation was detected. When the cells were incubated at 37°C after heat stress, the ratio between free SUMO-2/3 and SUMO-2/3 conjugates returned to their basal states, suggesting that a substantial pool of free SUMO-2/3 is maintained by reversible modification via SUMO proteases. Other studies of SUMO-2/3 have established that the expression of tumor suppressor ARF recruits SUMO-2 into the nucleolus and activates the sumoylation of various nucleolar proteins such as Werners helicase (301), p53 (39), Mdmx (213), and nucleophosmin (NPM1) (96, 271). SUMO-3 was not examined in the study of Haindl, (96), and it is not known whether ARF would have the same effect on SUMO-3.

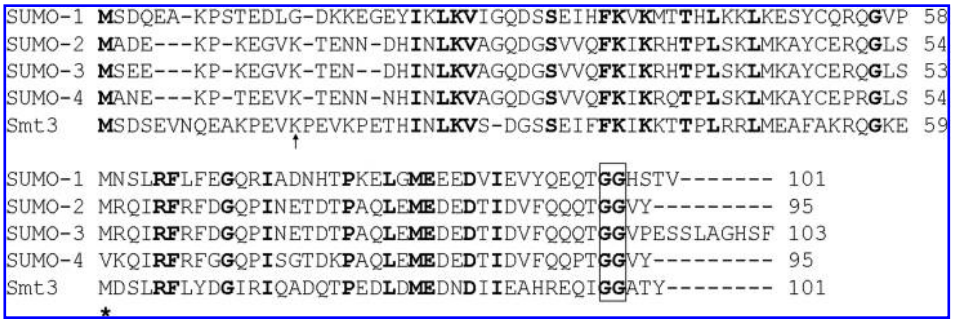

FIG. 1. Multiple protein sequence alignment of human SUMO isoforms and yeast Smt3. Conserved residues are in **boldface**. The Gly–Gly motif recognized by SUMO specific protease for the maturation process is *boxed*. The sumoylation site in SUMO-2/3 and Smt3 for polySUMO chain formation is indicated by an *arrow*. Substitution of a methionine by a valine at residue 55 in SUMO-4 is indicated by an *asterisk*.

C. SUMO conjugation system

As a ubiquitin-related post-translational modification, SUMO conjugation shares a similar pathway with ubiquitination, including a cascade of conjugation catalyzed by E1, E2 and E3 ligases (Fig. 2). However, unlike the ubiquitination pathway which is coordinated by a large number of E2s and several hundred substrate-specific E3, sumoylation involves only a heterodimeric E1, a single E2, and a few E3s. Prior to SUMO conjugation, synthesized SUMO undergoes maturation catalyzed by SUMO specific proteases (see Section III) to expose the C-terminal di-glycine motif. E1 activating enzyme is a heterodimer composed of Uba2 and Aos1 which forms a thioester linkage between an active cysteine of E1 and the C-terminal glycine of SUMO in an ATP-requiring process (123). Very little is known about the specific function of the individual subunits Uba2 and Aos1. However, Uba2 is a SUMO substrate and contains several SUMO attachment sites. After activation, SUMO is transferred to E2 conjugating enzyme (Ubc9) and forms a SUMO-E2 thioester linkage at an active cysteine residue of Ubc9. This intermediate acts as the SUMO donor in the conjugation reaction, in which SUMO is covalently linked via an isopeptide bond between its C-terminal carboxyl group at the di-glycine motif and the ε-amino group of a specific lysine in the substrate (242, 262). The almost common consensus sequence is ψKXE, in which ψ represents a hydrophobic residue and X can be any residue (242). This minimal core motif is found in over one-third of all characterized SUMO substrates. From the crystal structure of the Ubc9-SUMO complex, residues surrounding the active cysteine residue of Ubc9 seem to be capable of identifying the sumoylation consensus sequence of the substrate (17). Although *in vitro* sumoylation can be achieved by including E1 and E2 enzymes in the reaction mixture (59), distinct types of SUMO E3 ligase serve to promote the transfer of SUMO from Ubc9 to specific substrates (125). Three types of SUMO E3 ligases have been identified: PIAS family (protein inhibitor of activated STAT with divergent RING-like motif) (103, 211, 237, 243), RanBP2 (nuclear pore complex (NPC)-associated protein with zinc finger domain) (54, 145, 187, 224), and Pc2 (polycomb group protein) (128, 129) (Fig. 3). The PIAS family, consisting of PIASα, PIASβ, PIAS1, PIAS3, and PIASy, promotes sumoylation that resembles the action of RING-type ubiquitin E3 ligases (211). A number of studies have suggested that different E3 ligases catalyze the conjugation of a specific set of transcription factors through distinct mechanisms (130, 187, 251, 260). For example, PIASy triggers sumoylation and transcriptional activity of p53 (21) while RanBP2 is involved in Sp100 and HDAC4 modification (145, 224), and Pc2 acts on the transcriptional corepressors CtBP and CtBP2 (128). This discrepancy in substrate specificity is very likely related to the distinct intracellular localization of the SUMO conjugating enzymes and SUMO ligases (83, 128, 129, 224, 233, 237).

III. Sumo-Specific Proteases (SENPs)

Two SUMO specific proteases, Ulp1 and Ulp2, were first discovered in yeast (160, 161). Ulp1 is essential for the G2/M transition in the cell cycle and inactivation of *Ulp1* in *S. cerevisiae* is lethal (160, 273). Ulp1 is located at the nuclear pore complex and functions in both maturation and deconjugation (217). Ulp2 is localized predominantly in the nucleus, and its desumoylation pattern is distinct from that of Ulp1, but it is dispensable for viability (160).

To date, six SUMO proteases, SENP1 to 3 and SENP5 to 7, have been identified in humans (131, 142) (Fig. 4). Structurally, the less conserved N-terminal domain of the protease is responsible for cellular localization and substrate specificity during deconjugation (162). Although all SENPs share a conserved C-terminal catalytic domain with $\sim$250 amino acid residues, the domain of SENP1 shows a higher homology to that of SENP2 (59% homology) than to those of SENP3 (42% homology) and SENP5 (44% homology). According to the sequence homology, the SENP family can be divided into four subfamilies. SENP1 and SENP2 share a conserved sequence of $\sim$40 residues preceding their catalytic domain and constitute the first SENP1 subfamily. The catalytic domain of SENP3 is 62% identical to that of SENP5, indicating that SENP5 is more

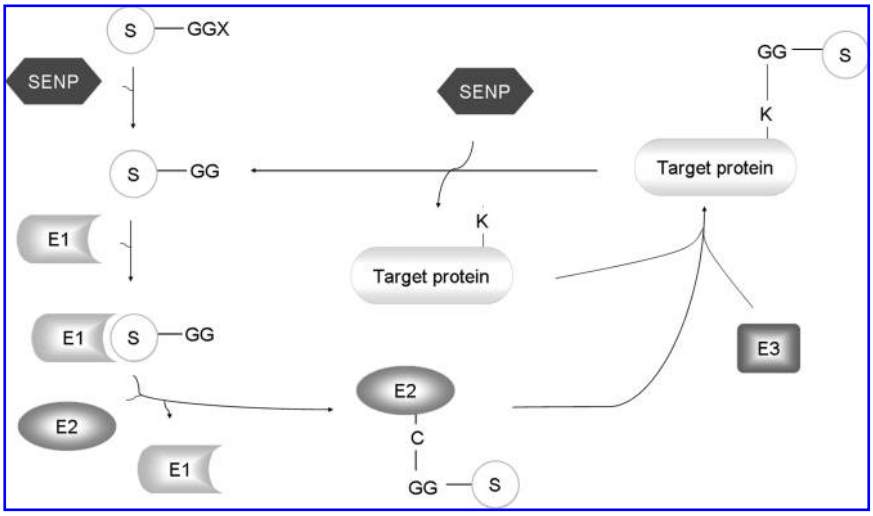

FIG. 2. SUMO conjugation/deconjugation system. Maturation of SUMO molecule is catalyzed by the SUMO specific proteases, SENP. After maturation, the exposed second glycine forms a covalent bond with the ε-amino group of a substrate lysine residue by a cascade of SUMO E1, E2 and E3 ligases. By using adenosine triphosphate (ATP), E1 enzyme activates SUMO by forming a thioester linkage between an active cysteine of E1 and the C-terminal glycine of SUMOs. After activation, SUMO is transferred to E2 by the formation of SUMO-E2 complex via thioester linkage between the active cysteine of E2 and C-terminal of SUMO. With the E3-like ligases, E2 further passes SUMO to its substrate. The conjugated protein has its function and/or localization altered to mediate downstream effects of sumoylation. The system is reversible by detaching SUMO from target proteins with SENPs.

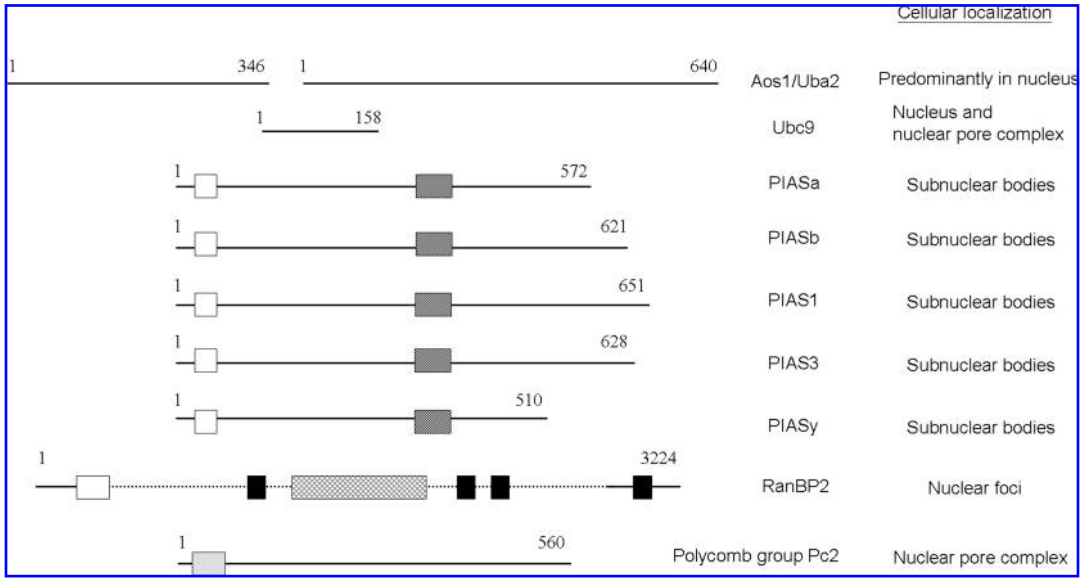

FIG. 3. Human SUMO conjugation enzymes. All PIAS family members consist of a DNA-binding domain (*open box*) and zinc finger motif (*striped box*). RanBP2 is featured with one TPR domain (*gray box*) and the eight zinc finger motifs are interspersed between residues 1355-1849 (*cross-hatched boxes*). The binding surface of Ran is indicated as (*black box*). Polycomb group Pc2 consists of a chromatin organization modifier (chromo) domain (*dotted box*) at the N-terminal.

closely related to SENP3. These two SENPs are therefore categorized into the second subfamily. The third subfamily of SENPs consists of SENP6 and SENP7, both of which contain an insertion of ~80 residues in their conserved catalytic domain. The fourth subfamily has only one member, SENP8 (105). Although SENP8 also possesses the SENP catalytic domain, it hydrolyzes NEDD8 conjugates instead of SUMO conjugates. Analysis of the SUMO-binding sites on SENP2 indicates that the side chains of R456 and W457 of SENP2 interact with the conserved SUMO-1. However, in the SENP8 sequence, these two residues are substituted with Met and Phe, respectively (231). Phylogenetic analysis indicates that Ulp1 is related to SENP1, SENP2, SENP3, and SENP5, while Ulp2 is related to SENP6 and SENP7.

As mentioned above, SENPs catalyze the formation of mature SUMO for conjugation and removal of SUMO from the conjugated substrates. The *in vitro* endopeptidase activity in maturation and isopeptidase activity in deconjugation of individual SENPs (except SENP3) have been characterized (70, 186). Using SUMO precursors and sumoylated RanGAP1 as substrates, it has been found that SENP1 exhibits the most efficient endopeptidase activity, while the other SENPs show substantially higher isopeptidase than endopeptidase activity. Nevertheless, an increasing number of findings on the expression of SENPs in different carcinoma tissues or cell lines indicates that it is vital to achieve a proper balance of sumoylation and desumoylation. It has been shown that gene disruption of SENP1 due to chromosomal translocation with

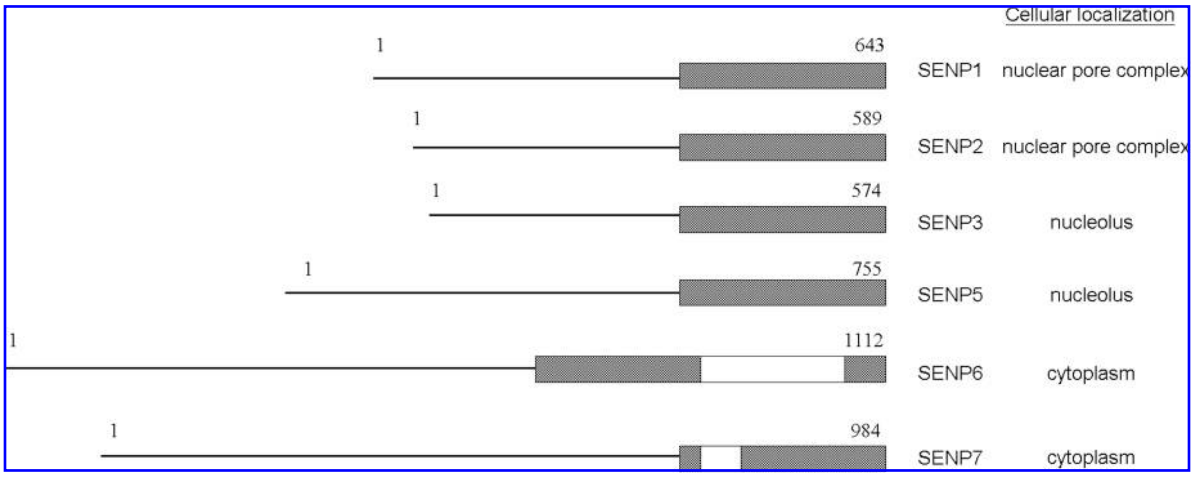

FIG. 4. Human SUMO specific proteases. The N-terminal nonconserved domain is associated with subcellular localization and substrate specificity. The C-terminal conserved catalytic domain is shown as a *hatched box*. Insertion of ~180 and 50 amino acid residues in the catalytic domains of SENP6 and SENP7 is shown as an *open box*.

MESDC2 (an endoplasmic reticulum protein related to embryonic polarity) is associated with infantile teratoma (286). Interruption of SENP6 by fusion to TCBA1 (T cell lymphoma breakpoint associated target 1) is observed in the human T cell lymphoma cell line, HT-1 (270).

A. Ulp1/Ulp2

The yeast protease Ulp1 was the first identified SUMO protease, targeting Smt3 and SUMO-1 but not ubiquitin (160). Yeast two-hybrid screening and co-immunoprecipitation experiments have shown that Ulp1 interacts with nuclear pore complex (NPC) or NPC associating components (273). Further investigations of *Schizosaccharomyces pombe* indicate that Ulp1 is localized to the nucleus in a cell cycle-dependent manner, in that it is located close to the nuclear periphery during the S and G2 phases but is within the nucleus in anaphase (277). The association of Ulp1 and nucleoporins accounts for DNA maintenance through regulating the sumoylation of several cellular proteins, including the DNA repair factor Yku70 (210). Overexpression of the catalytic domain of yeast homolog Ulp1 is lethal in yeast, suggesting that the unbalanced desumoylation may be toxic (191). Although opinions differ as to whether the lack of the Ulp1 gene is lethal, deletion of Ulp1 can definitely cause severe cell and nuclear abnormalities (66, 277). As with all other SUMO proteases, Ulp1 contains a catalytic C terminal domain and a noncatalytic N terminal domain. The N-terminal domain of Ulp1 contains two distinct binding sites for anchoring to NPC, and therefore excluding Ulp1 from the nucleoplasm for cell viability (217). The importance of the N-terminal segment in substrate specificity is evidenced by the accumulation of SUMO conjugates when only the C-terminal domain of Ulp1 is expressed. In contrast to the full length of Ulp1, the C-terminal of Ulp1 can suppress defects of cells lacking Ulp2, indicating that the N-terminal domain of Ulp1 regulates and restricts certain isopeptidase activities of Ulp1 (162).

Ulp2 is located in the nucleoplasm, and was first isolated as a high-copy-number suppressor of a defective centromere-binding protein (161). It also functions as an SMT3 deconjugating enzyme, but its desumoylation pattern is distinct from that of Ulp1. Interestingly, the available genomic sequences of *S. cerevisiae* and *S. pombe* indicate that the active site of Ulp2, unlike other SUMO proteases, consists of a serine residue instead of a cysteine residue. However, the biological significance of a SUMO serine protease remains to be discovered. Notably, high-molecular-weight SUMO-containing species accumulate in Ulp2 mutant, suggesting that Ulp2 is able to cleave SUMO chains (32). Ulp2 is also found to resume the cell cycle after DNA repair and mutation of Ulp2 causes escape of the checkpoint-induced metaphase arrest (244).

B. SENP1

SENP1 was first cloned by E.T.H. Yeh's group (87). It is localized mainly in the nucleus, excluding the nucleolus. A single nonconsensus nuclear localization signal (NLS) is positioned within its N-terminal. Mutations at the N-terminal fragment result in the cytoplasmic accumulation of SENP1 (10). In addition to NLS, SENP1 also features a distinct nuclear export signal (NES) in the C-terminal region, thus displaying a nucleocytoplasmic shuttling property (144). Interestingly, in herpes virus, though a sumoylation system is lacking, its regulatory protein ICP0 promotes and co-localizes with SENP1 in the nucleus, therefore affecting the desumoylation profile, including the SUMO-1 modified species of PML (9). Recently, the trafficking of SENP1 has also been shown to be regulated by tumor necrosis factor (TNF) and radical oxygen species (ROS). SENP1 interacting with an antioxidant protein thioredoxin is localized in the cytoplasm in resting endothelial cells. Nuclear import of SENP1 is promoted by TNF via the release of SENP1 from thioredoxin. However, this TNF-induced translocation can be blocked by antioxidants, such as N-acetyl-cysteine (165).

The targeting substrates of SENP1 include a large family of transcription factors and their co-regulators such as androgen receptor and histone deacetylases (13, 40). SENP1 is also responsible for the processing and deconjugation of SUMO-1. Cells deficient in SENP1 accumulate both unprocessed SUMO-1 and SUMO-1 conjugates, while SUMO-2/3 and their conjugates remain (309). The biological significance of SENP1 is closely associated with development, as shown in a number of transgenic studies. Transgenic mice with reduced expression of SENP1 show placental abnormalities and the embryos are not viable (87). Mouse embryos with SENP1-/- genotype develop severe fetal anemia and die in midgestation (42). It has also been reported that SENP1 modulates the stability of hypoxia-inducible factor 1α (HIF-1α) during hypoxia and controls the production of erythropoietin for differentiation of red blood cells. On the other hand, overexpression of *Xenopus* SENP1 inhibits embryonic dorso-anterior development and suppresses transcription activation in the Wnt signaling pathway (316). The clinical relevance of SENP1 has recently been demonstrated in human prostate cancer. SENP1 is overexpressed in 60% of cases of high-grade prostatic intraepithelial neoplasia and prostate cancer (41), In contrast to the other SENPs, SENP1 dramatically enhances the androgen receptor(AR)-mediated expression of genes regulating cell growth, apoptosis and transformation in prostate cancer cells, Pc-2 (13, 41).

C. SENP2

SENP2 is a nuclear envelope-associated protease, found in the nucleoplasmic face of the nuclear pore complex (97, 318). Like SENP1, it also contains an NLS and NES and behaves as a nucleocytoplasmic shuttling protein. It is susceptible to cytoplasmic ubiquitination and proteasomal degradation (118), though the regulation and biological consequences of this degradation remains imperfectly understood. A mouse homologue, SUSP4 (also known as SMT3IP2/Axam2), is found in association with p53 and mdm2. It has been shown that deconjugation of mdm2 by SUSP4 promotes mdm2 self-ubiquitination and degradation, and ultimately positively modulates p53 response (155). An alternative spliced form of mouse SENP2 (SuPr1) has also been reported, which regulates Sp3 activity and alters the distribution of nuclear promeylocytic leukemia (PML) oncogenic domain-associated proteins such as CBP and Daxx (87). SENP2 clearly has some biological significance, as evidenced by its interaction with Axin and its augmentation of β-catenin degradation in the Wnt signaling pathway (127, 293, 310).

D. SENP3

Both SENP3 and SENP5 are localized in the nucleolus and show a preference for desumoylating SUMO-2/3 conjugates

(63, 88, 205). Their distinct localization in the nucleolus may imply that they are associated with preribosomal processing modification and assembly of mature ribosome subunits through eliminating the SUMO-2/3 modification of nucleolar proteins while allowing SUMO-1 conjugates to persist. An exploration of the functional importance of SENP3 has recently been made (96). Immunoprecipitation using an inactive SENP3 was employed to isolate a number of ribosomal subunit proteins. The major interacting partner was nucleophosmin (NPM1), which is required for the processing of 32S rRNA to 28S rRNA during ribosome biogenesis. It has been proposed that tumor suppressor ARF interferes with the function of NPM1 and inhibits the maturation of the 28S rRNA (61, 90). Using the yeast two-hybrid system, the interaction between SENP3 and NPM1 has been shown to be specific to SENP3, but not to SENP1, SENP2, or SENP5. Expression of ARF, which is localized in the nucleous, can induce SUMO modification of NPM1 which subsequently prevents 28S rRNA maturation. Intriguingly, expression of SENP3 causes a complete loss of ARF-induced SUMO-2 conjugated NPM1 but not of the SUMO-1 conjugated form, while depletion of SENP3 inhibits rRNA processing. These results suggest that SENP3 counteracts the tumor suppressor ARF-induced modification of NPM1 and is an essential factor for ribosome biogenesis. To date, five other subsets of heterogeneous nuclear ribonucleoproteins have been shown to be modified by SUMO (hnRNP C and hnRNP M proteins, and hnRNP A1, hnRNP F, and hnRNP K) (163, 284). Further studies are required to ascertain whether SENP3 is also involved in the other RNA processing events.

E. SENP5

Tissue distribution by immunoblotting in different cell lines demonstrates that SENP5 is present predominantly in the prostate (PC3 cells), blood (HeLa cells), and breast (MCF7 cells) (88). The exclusively nucleolar localization of SENP5 is due to the presence of the N-terminal region, as truncated SENP5 lacking the first 168 residues is localized in the nucleus and cytosol. Although SENP5 shows a preference for desumoylating SUMO-2/3, using various sumoylation defective PML mutants, reports suggest that its activity is not restricted to SUMO-2/-3. Its ability to deconjugate SUMO-1 on PML is limited to the Lys 65 site, but not to the Lys 160 and Lys 490 sites, suggesting that specificity towards SUMO deconjugation is also conferred by the SUMO attachment position. As far as its hydrolase activity is concerned, the deletion of a 571-residue fragment from the N-terminal does not affect its protease activity, but the additional removal of a 29-residue fragment does. This implies that the catalytic activity of SENP5 is solely conferred by the catalytic domain. A recent study has demonstrated that SENP5 is also localized in the cytosol, and that the overexpression of SENP5 in COS-7 cells reduces the level of SUMO-1 conjugates–specifically a SUMO substrate, dynamin related protein (DRP1)–in the mitochondria (327). The recruitment of DRP1 from cytosol to the mitochondrial outer membrane is necessary during mitochondrial fission. Silencing of SENP5 stabilizes DRP1 and leads to a fragmented and widened mitochondrial morphology, suggesting that SENP5 is involved in mitochondrial morphology and metabolism. Although SENP3 and SENP5 are similar in sequence and biochemical features, they are not functionally redundant. Suppression of SENP5 expression has resulted in the inhibition of cell proliferation and development with an altered nuclear architecture, suggesting that SENP5 is crucial in mitosis and/or cytokinesis (63).

F. SENP6 (SUSP1) and SENP7

The proteases SENP6 and SENP7 are found in the cytosol (141, 192) and are closely related to the yeast Ulp2. A recent study of SENP6 has discovered that it is also located in the nucleus and displays a preference for cleavage of SUMO-2/3 polymeric chains. SENP6 and SENP7 display similar hydrolytic activities to SENP2 in SUMO-2/3 deconjugation reactions and cleavage reactions of SUMO-2/3 dimer/polymer, but much lower activities for SUMO-1 deconjugation reaction. In contrast to SENP2, which plays a functional role in processing SUMO precursors, SENP6 and SENP7 showed no detectable activity in SUMO maturation reaction (166, 186). The siRNA-mediated depletion of SENP6 indicates that it might play a role in PML body maintenance (192). SENP6 has also been found to control the transcriptional activity of RXRα, which belongs to a family of ligand-activated transcription factors regulating metazoan life. SENP6 is co-localized with RXRα in the nucleus and counteracts SUMO-1 conjugation with RXRα, causing an increase in the transcriptional activity of RXRα (45). The crystal structure of SENP6 and SENP7 has recently been solved, providing some valuable information about this most distinct class of SUMO proteases (166). However, the full extent of the biological functions of these two SUMO proteases, and their physiological significance, have yet to be elucidated.

IV. Hydrolytic Functions of SUMO Proteases

A. SUMO maturation

All SUMO proteins are expressed in their precursor forms and are widely distributed among different tissues (177, 240, 249, 250, 306). Precursor processing involves cleavage of the residues after the C-terminal conserved 'GG' motif by a SUMO-specific protease. The exposed second glycine residue can thereafter form an isopeptide bond with lysine on SUMO substrates. Human SENP2 (141), yeast Ulp1 (191), and *Arabidopsis* ESD4 (197) were the first SUMO proteases reported to be functional in SUMO maturation. Several lines of evidence later demonstrated that the catalytic domains of SUMO proteases exhibit different maturation efficiencies to different SUMO precursors. SENP2 has been shown to contain the highest maturation efficiency to SUMO-2 (230), while the processing efficiency of SENP1 follows the order SUMO-1> SUMO-2> SUMO-3 (306). It is clear from the sequence alignment of SUMO precursors that SUMO paralogues are highly conserved, especially those for SUMO-2 and SUMO-3 (86%). By mapping the residues of SUMO precursors, the difference of the sequence after the Gly–Gly motif of SUMOs has also been shown to influence maturation efficiency (186, 306). In contrast to SENP1 and SENP2, SENP5 only processes SUMO-3 maturation (88). Since the maturation reaction is the first committed step for subsequent sumoylation, the different maturation efficiencies catalyzed by different SUMO proteases may regulate the availability of different SUMO proteins for conjugation. In particular, when taken together with the results from Mikolajczyk (186) showing that SENP1 is the most efficient

endopeptidase, it is very likely that SENP1 is the key player regulating the availability of 'free' SUMO-1 for conjugation.

B. SUMO deconjugation/depolymerization

Sumoylation is a dynamic process that is reversible by removing the SUMO moiety from the substrates by SUMO-specific proteases. This is necessary to control the half-lives of SUMO conjugates and regulate their effects on downstream cellular events (101), while the SUMO moiety released can be recycled for another conjugation process. The deconjugation process involves cleavage of the amide bond between the carbonyl group of the C-terminal glycine in SUMO and the ε-amino group of the target lysine in the conjugate. The importance of deconjugation has been demonstrated by investigating the biological effects of substrates upon conjugation and deconjugation. The human DNA repair protein, thymine-DNA glycosylase (TDG), was previously identified as a SUMO substrate. Upon completion of the base excision process, SUMO conjugation occurs and alters the structural conformation of TDG, dramatically reducing its affinity to abasic DNA product (7, 98, 124). To provide the unconjugated form which possesses a strong DNA binding activity for another repairing cycle, SUMO deconjugation is required. Another well-characterized example is K2P1, an integral cytoplasmic membrane channel-forming protein. The activity of K2P1 is totally abrogated after sumoylation on the cytoplasmic fragment, until desumoylation (227). Most of the native K2P1 is sumoylated under normal conditions. This explains the long-standing observation of the inability of this channel to function despite its abundant mRNA expression in the heart, brain, and kidney. Notably, desumoylation modulates K2P1 to a K+-selective, pH-sensitive openly rectifying channel. SUMO protease has also been found to regulate gene transcription through PML (323). The PML protein facilitates the assembly of the PML bodies for which the PML protein must be sumoylated. SUMO protease SuPr-1 has been found to hydrolyze SUMO-1 conjugated PML and alter the subcellular distribution of PML in nuclear PODs (PML oncogenic domains) (18).

The depolymerization process is chemically equivalent to that of deconjugation, but instead of cleavage of the isopeptide bond between the C-terminal glycine of SUMO and the ε-amino group of target lysine of the substrate, target lysine in SUMO-2/3 or in yeast Smt3 is involved. Characterization of budding yeast Ulps knockout mutants shows that depletion of Ulp2 causes accumulation of high-molecular-weight SUMO conjugates (32). This phenotype is only found when the cells express wild-type Smt3 capable of forming a polymeric chain, but not the Smt3 mutant having a substitution in the target lysine residue. The results suggest that Ulp2 family members actively participate in the depolymerization process. An evolutionary conservation analysis suggests that SENP6 and SENP7 are responsible for dismantling the SUMO polymers. Interestingly, downregulation of SENP6 in mammalian cells only shows subtle changes in the level and distribution of SUMO-2/3 conjugates, suggesting that SENP6 and SENP7 may be redundant enzymes (192).

C. Implications from the crystal structures of SENPS, SENP/SUMO, and SENP/SUMO conjugated complexes

All six SUMO proteases possess a conserved C-terminal domain, although these are variable in size and have a distinct N-terminal domain. The C-terminal domain exhibits catalytic activity, while the N-terminal domain regulates cellular localization and substrate specificity (142, 144). Mossessova and Lima (191) first determined the crystal structure of the covalent thiohemiacetal transition state complex formed between a budding yeast Ulp1 C-terminal fragment and Smt3 (Fig. 5). The Ulp1 structure (residues 403–621), consisting of seven α-helices and seven β strands, has a significant structural similarity to adenoviral protease-1 (AVP1) (64) and resembles those of others in the cysteine protease superfamily (160). In particular, the structure shares similar features with other cysteine proteases in the active site and the catalytic triad (Cys-His-Asp) (71). For the Smt3 structure, it is composed of five antiparallel β strands and one α-helix. Smt3 does not undergo large conformational changes when complexed with Ulp1. The N-terminal residues 1–18 are disordered but the C terminus fragment adopts an extended β conformation that ends with a covalent bond between Gly-98 and the protease active-site cysteine nucleophile (Cys-580). From the Ulp1/Smt3 complex, six conserved structural motifs directly involved in Smt3 binding and peptide hydrolysis have been identified (Fig. 6). Motifs 1 (residues 432–438) and 4 (residues 489–493) form the respective sides of the Ulp1-Smt3 interacting interface, while motifs 2 (residues 448–456), 3 (residues 472–478), and 5 (residues 509–515) cluster in a patch to recognize the Smt3 β sheet and C-terminal β-strand ending with the Gly-Gly motif. Motif 6 (residues 574–580) consists of several conserved Ulp1 active-site residues. The catalytic site is located in a shallow and narrow cleft in which conserved amino acids interact with the Smt3 Gly-Gly-X motif. Remarkably, the Gly-Gly motif, once inserted, is clamped within a hydrophobic tunnel by the conserved tryptophans (Trp 448 and Trp 515) and several conserved residues nearby (Cys 580, Ser 513, and His 514). Mutation of the Gly-Gly motif of Smt3 generates a steric clash within the tunnel and completely abolishes the interaction with the SUMO protease. The structures also help to explain why SUMO-4 cannot be processed. Residue Gln 95 located at the N-terminal of the diglycine motif in SUMO-1 is essential for interaction with the protease. Substitution with a Pro90 in SUMO-4 would alter the orientation of the neighboring Gly-Gly motif and prevent its interaction (209).

For cysteine proteases, the nucleophilic active-site cysteine is coordinated by a general base histidine residue, which is in turn stabilized by an aspartate residue. A comparison of SENP2 with the SENP2–SUMO-1 complex demonstrates that SENP2 undergoes local structural rearrangements in response to SUMO-1 binding (230) (Fig. 7a). This includes a reorientation of His 478 towards Asp 495 at the catalytic triad, and rotation around the Cβ of Trp410, Trp479, and His 474 (in SENP2 sequence). Interestingly, comparison with the SENP1–SUMO complex structures (253, 307) shows that only part of this rearrangement of Trp 465, His 529, and Trp 534, which is induced by SUMO binding, is observed. Instead, His 533 at the active site forms a hydrogen bond with the hydroxyl group of Ser 603, which mimics the active cysteine residue. The results suggest that this structural rearrangement at the SUMO protease active site enhances the hydrolysis reaction but not the release of cleaved SUMO.

Two recent independent studies have provided further information on the atomic details of the cleavage mechanism by the crystal structures of catalytically inactive SUMO

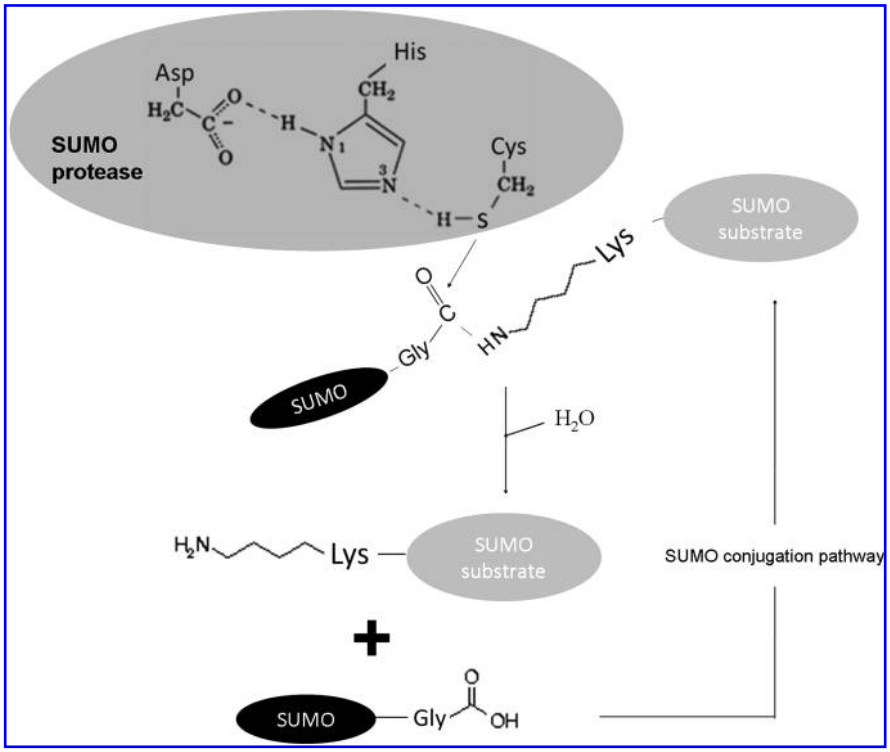

FIG. 5. The molecular mechanism of SUMO deconjugation reaction. In the catalytic triad of SUMO proteases, the active-site cysteine is coordinated by a general base histidine residue, which is in turn stabilized by an aspartate residue. During the SUMO deconjugation process, the active nucleophilic cysteine attacks the carbonyl carbon of the isopeptide bond to initiate the hydrolysis reaction. The cleaved SUMO can be recycled for SUMO conjugation reaction.

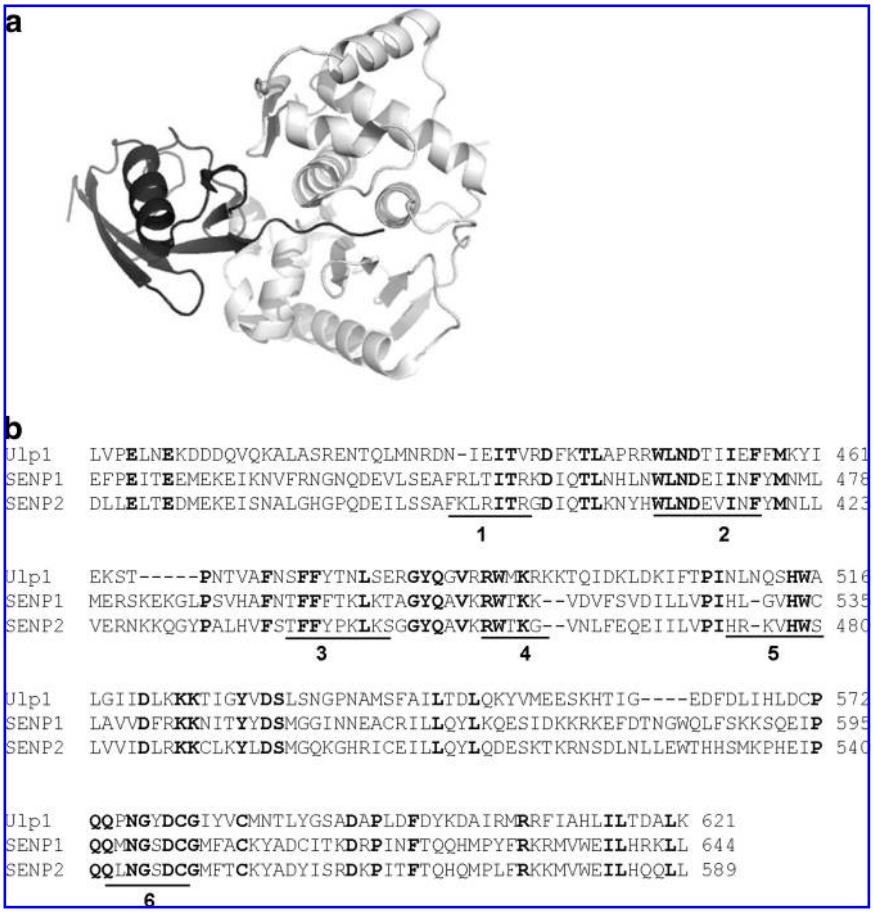

FIG. 6. (a) Crystal structure of Ulp1/Smt3 complex. Ribbon diagram of the crystal structure of Ulp1/Smt3 complex. Ulp1 and Smt3 are colored in *white* and *dark gray*, respectively. (**b**) Multiple sequence alignment of Ulp1 and SENP1. The six motifs for the interaction of Ulp1 and Smt3 are *underlined* and *labeled*. Conserved residues are in **boldface.**

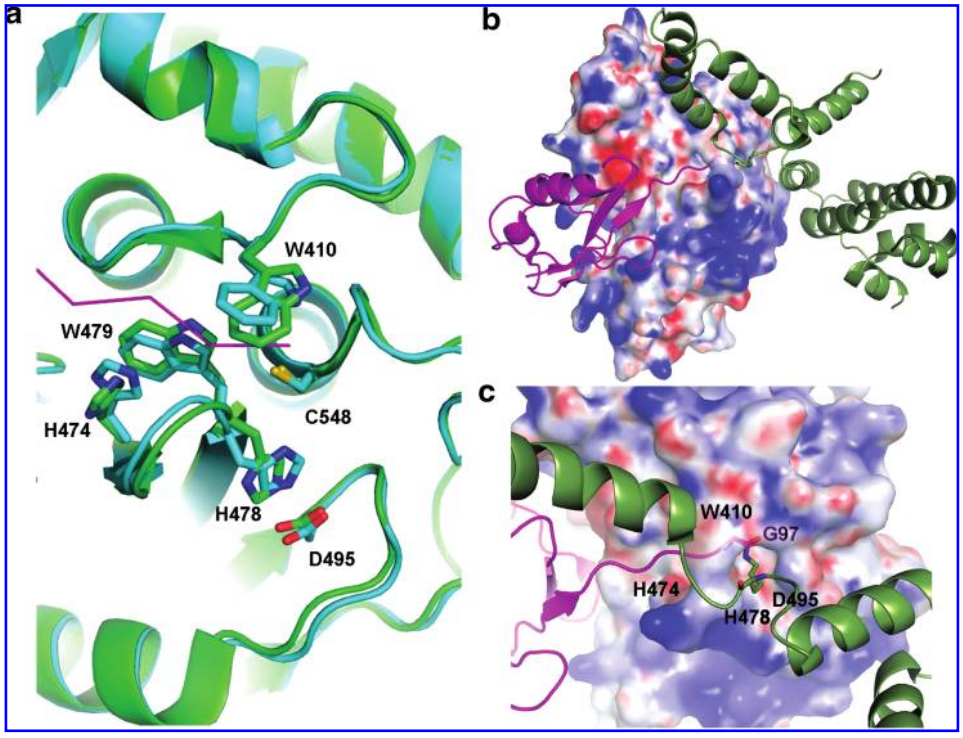

FIG. 7. (a) Structural superimposition of SENP2/SUMO-1 and SENP2 apoenzyme. SENP2 in SENP2-SUMO1 complex is shown in *green* and SENP2 apo enzyme is shown in *cyan*. When binding with SUMO1 (*purple*), the catalytic triad of SENP2 undergoes a local rearrangement (C548, H478, and D495). To facilitate binding with SUMO-1, W410, W479, and H474 also have conformational changes. (**b**) Crystal structure of SENP2/SUMO-1 conjugated RanGAP1. Surface representation of SENP2 and its interaction with sumoylated RanGAP1. Surface oxygen and nitrogen atoms of SENP1C are colored in *red* and *blue*, respectively. SUMO-1 conjugated RanGAP1 is shown in ribbon mode and SUMO-1 is and RanGAP1 are colored in *magenta* and *dark green*, respectively. (**c**) The scissile isopeptide bond of the C-terminal glycine of SUMO and the targeted lysine residue of RanGAP1. The scissile isopeptide is kinked at 90 degrees and adopts a cis configuration of the amide nitrogens. Color labels of residues are in accordance with their respective protein molecules. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

proteases bound to SUMO precursors or sumoylated substrate, RanGAP1 (232, 253, 254). Shen *et al*. (253, 254) determined the complex structures of SENP1 bound to SUMO-1 modified RanGAP1, and SENP1 complex with SUMO-1 precursor. In both cases, the scissile isopeptide bond of the C-terminal glycine of SUMO and the lysine side chain is kinked at 90 degrees and adopts a cis configuration of the amide nitrogens (253, 254) (Fig. 7b and c). Identical implications are also drawn from the four complex structures of SENP2 complex with RanGAP1–SUMO-1, RanGAP1–SUMO-2, SUMO-2 precursor, or SUMO-3 precursor (232). A similar kinked scissile isopeptide bond is observed for all four structures. Biochemical analysis also suggests that the discrimination between SUMO-1 and SUMO-2 maturation by SENP1 (306) is based on a catalytic step (k_{cat}) rather than substrate binding (K_m) (253, 254). Taking together, the previously found structural rearrangement at the catalytic domain of SUMO proteases and the newly found kink at the scissile isopeptide bond, the authors further proposed two steps for the hydrolytic reactions for SUMO processing and SUMO deconjugation. First, the SUMO proteases bind with SUMO precursors/sumoylated substrates and induce the opening of the tryptophan tunnel. Second, closure of the tryptophan tunnel causes trans–cis isomerization of the amide nitrogens of the scissile bond of the SUMO precursors or substrate (253, 254). These new findings have shed valuable light on the mechanism of discrimination between SUMO paralogues during maturation processing and SUMO deconjugation by SUMO proteases. From the structural information, the poor endopeptidase activity of SENP6 and SENP7 is possibly due to the replacement of the critical Trp 410 (SENP2 sequence) by Phe (186). Residue Trp 410 acts as a flap that directs the entry of the Gly-Gly motif of SUMO to the catalytic pocket. Mutation of Trp 410 to Ala completely abolishes its SUMO precursor processing activity (232), indicating that Phe substitution in SENP6 and SENP7 prohibits the endopeptidase. However, the underlying mechanism for the retention of isopeptidase activity remains unclear.

The crystal structure of the catalytic domain of SENP7 has recently been determined (166). Compared with the current available structures of SUMO proteases, SENP7 does not align well to SENP1 and SENP2. SENP7 possesses unique structural elements, in particularly four insertion loops (residues 685–692, 748–767, 811–861, and 940–956, respectively) (Fig. 8). Loops 1 and 2 are in the close proximity of the SUMO binding surface whereas Loops 3 and 4 are located on the opposite surface of the catalytic domain. The biological significance of these loops has been studied by constructing various mutants. The mutant SENP7 with Loop 1 (685–692) deletion displays reduced enzymatic activities in deconjugation and cleavage of SUMO-2/3 dimers or polymers. Based on the structural modeling of SENP7 and SUMO interaction, Loop 1 is likely to project to the binding surface of SUMO substrates. This would explain how it can significantly affect the hydrolytic activities of SENP7. In addition, residue Val 713, which is positioned adjacent to Loop-1 and may interact with SUMO2/3 by modeling, is critical to the hydrolytic activities of SENP7. By structural alignment, the position of V713 is equivalent to a conserved glutamate in SENP1 and SENP2, and is at the interface with SUMO in SENP-SUMO complex structures (222, 244, 292). The mutant V713E shows much lower hydrolytic activities than the wild type, probably due to the polar

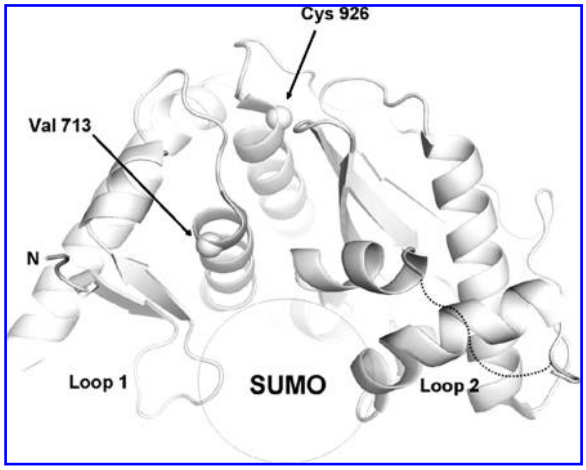

FIG. 8. Ribbon diagram of the crystal structure of the catalytic domain of SENP7. The two extended loops unique to SENP6/SENP7 are shown. Loop 2 is disordered and is drawn by *dotted line*. Loops 3-4 are positioned at the opposite side of the SUMO binding surface and are not shown. Val 713 essential for deconjugation of SUMO-2/3 and active-site Cys 926 are shown as *spheres*.

interactions with SUMO, and this suggests that SENP7-SUMO and SENP1/2-SUMO have their distinct SUMO interacting surfaces. It is very likely that SENP6 also carries a comparable molecular mechanism in SUMO recognition and catalysis.

V. Biological Significance of Sumoylation/ Desumoylation

With an increasing number of SUMO conjugates identified (148, 163, 206, 245, 287, 296) (Table 1), the next steps in studying this new post-translational modification system are to understand the molecular basis of modification and the biological consequences and implications. Most previous studies have demonstrated a direct relationship between SUMO ligases and the substrates. However, it is undoubtedly explicable that SUMO proteases regulating the reverse of the modification would have an equal significance (Fig. 9).

A. Transcription expression

In many cases, the prominent effect of sumoylation is to suppress transcription, either by enhancing the activity of repressors or co-repressors or by inhibiting the activity of transcription activators. However, an increasing number of investigations have shown that sumoylation can also activate transcription. Nevertheless, removal of SUMO from conjugates by SENPs would oppose the effect of sumoylation. One of the well-characterized substrates in sumoylation essential in gene transcription is PML. This potent tumor suppressor, localized in discrete subnuclear compartments known as nuclear bodies (NBs), PML oncogenic domains (PODs), or nuclear domain 10 (ND10), is essential for the assembly of these subnuclear structures. PML in NBs complexes with and modifies the activity of a wide spectrum of transcription factors and regulators in DNA repair, genome stability, viral infection apoptosis, and tumor suppression (33, 79, 201, 265). SUMO modification of PML is necessary for NB formation

Table 1. SUMO Protein Substrates Identified in Mammalian Cells and Viruses

Transcription factors/regulators
AIB1, AP-2, APA-1, AR, ARNT, c-Jun, c-Myb, CBP, CREB, CtBP1, Elk-1, GATA-2, GR, GRIP1, HDAC1, HDAC4, HIF1α, HSF-1, HSF-2, Huntingtin, IRF-1, KLF8, Lef-1, LXR, MBD, Mdm2, MEF2, MKL1, Msx1, p300, p53, p73a, Pdx1, PLZF, PR, RBP1, Reptin, RXRα, Sam68, Smad4, SnoN, Sox2, Sox6, Sox10, Sp1, Sp3, SREBP, SRF, Stat1, Stat4, Tef4
Nuclear bodies
Daxx, HIPK2, PML, Sp100, TEL
Ribonuclear proteins
hnRNP A1, hnRNP C, hnRNP F, hnRNP K, hnRNP, NPM1
DNA replication, recombinant and repair
BLM, TDG, PCNA, TDG, WRN, XRCC4
Genome integrity and stability
Cenp-C, Dnmt3a, Dnmt3b, Pds5p, Rad52, TopI, Top II
Nuclear pore complex
RanGAP1, RanBP2
Cytoplasmic proteins
APP, Axin, Caspase8, DRP1, FAK, GLUT1, GLUT4, IκBα, NEMO, PDGFc, Phosducin, PTP1B, SOD1, Tau, Tax
Membrane proteins
Fas, GluR6, K2P1, mGluR8, PPARγ2, TGFbI, TNFR1
Viral proteins
Adenovirus: Gam1, E1B-55K
Bovine papillomavirus: E1
Chicken anemia virus: apoptin
Cytomegalovirus: IE1, IE2, IE72
Epstein-Barr virus: Rta
Herpesvirus: IE1
Kaposi's sarcoma-associated herpesvirus: K-bZIP
SARSCoV: N
Tula hantavirus: N

and association with other transcription factors such as Daxx, HDAC1, p53, and LEF1 etc. (117, 122, 145, 149, 167, 169, 243, 298). Furthermore, SUMO-PML NBs function as dynamic molecular reservoirs, controlling the availability of certain transcription factors to active chromatin domains (36, 158) and modulating their interactions with NBs. On the other hand, the underlying mechanism of arsenic trioxide (As_2O_3) in the treatment of acute promeylocytic leukemia (APL) has been found to be closely related to the sumoylation of PML (103, 196, 325). As_2O_3 increases the level of phosphorylated PML via a mitogen activated protein kinase (MAPK) pathway, and its subsequent sumoylation restores NB assembly to induce apoptosis in APL cells.

B. Genome stability and DNA repair

A recent investigation has determined the biological relevance of RanBP2-directed TopoII in centromere during mitosis (54). During interphase, RanBP2 interacts with RanGAP1 and the complex is localized on the cytoplasmic face of the nuclear pore. Once mitosis is initiated, RanBP2-SUMO conjugated RanGAP1 and Ubc9 complex assembles with the nuclear export receptor Crm1 and behaves as a component of the kinetochore (6). Deletion of RanBP2 results in kinetochore–microtubule attachment and mislocalization of the kinetochore and spindle of assembly spindle checkpoint proteins (126). An investigation that lasted more than 2 years found

that transgenic mice with reduced expression of RanBP2 developed severe aneuploidy in the absence of overt transport defects (54) and were highly sensitive to tumor formation. Splenocytes and embryonic fibroblasts derived from these mice showed a chromosome segregation defect of anaphase-bridge formation. The defect was rescued by expression of RanBP2 E3 domain, suggesting that RanBP2 regulates Topo II by SUMO modification. The importance of sumoylation in genome stability has also been demonstrated by studying the heterochromatic silencing in fission yeast (256). It has been shown that E2 ligase, Hus5 associates with heterochromatin proteins Swi6 (homolog of heterochromatin protein 1), Chp2 (a paralog of Swi6), and Clr4 (methylates the Lys9 of H3), are highly enriched in heterochromatic regions in a heterochromatin-dependent manner.

For DNA recombination and DNA repair, the molecular effects of a number of associated SUMO substrates have been elucidated. For example, proliferating cell nuclear antigen (PCNA) is SUMO-modified during the S phase, resulting in the recruitment of helicase Srs2 that subsequently disrupts Rad51 nucleoprotein filaments, thereby inhibiting unwanted recombination (106, 218, 223). In homologous recombination, sumoylation of Rad52 is induced upon DNA damage and triggered by Mre11–Rad50–Xrs2 (MRX) complex-governed double-strand breaks. SUMO attachment sustains Rad52 activity and concomitantly shelters the protein from accelerated proteasomal degradation (106, 238). A more detailed molecular mechanism has been deduced from the crystal structure of thymine DNA glycosylase (TDG) conjugated to SUMO-1 (7). It is believed that sumoylation of TDG alters its structural conformation and facilitates its dissociation from the abasic DNA product after base excision (7, 98, 124). On the other hand, Mms21/Nse2 (a yeast SUMO E3 ligase) colocalized with structural-maintenance-of-chromosome proteins (Smc) in the nuclear foci is responsible for the sumoylation of Smc6 (5, 269, 320) to promote DNA repair (225). A defect of Mms21 in budding yeast results in increased sensitivity to DNA damage (320).

C. Nucleocytoplasmic trafficking

The most abundant sumoylated conjugate, RanGAP1, is localized in the nucleocytoplasmic face and can activate RanGTPase, which governs the nucleocytoplasmic trafficking of proteins. In addition to its role in centromere association during mitosis, sumoylation of RanGAP1 disrupts dimer formation of unmodified RanGAP1 that may allow interaction with protein in NPC (181). Another example of sumoylation-mediated intracellular trafficking is demonstrated by ribosomal precursor particles that shuttle from the nucleous to the nucleoplasm and finally to the cytoplasm. The biological function of the sumoylation of these ribosomal particles is associated to the distribution of SENPs in the nucleolus (216). Intriguingly, many ribosome biogenesis factors are SUMO substrates. In particular, preribosomal particles along both the 60S and the 40S ribosomal synthesis pathways (96, 215, 235) are SUMO conjugates. Furthermore Ulp1, which is localized in the nuclear pore complex, is functionally linked to the 60S export factor Mtr2. Taken together, sumoylation of preribosomal particles in the nucleus and subsequent desumoylation at the NPC are essential for efficient ribosome biogenesis and export.

D. Signal transduction

The Wnt signaling pathway controls development and regulates cellular proliferation and differentiation (20, 248, 297). Constitutive activation of bipartite transcription factor β-caternin-TCF/LEF is frequently associated with colorectal cancer. Accumulating evidence supports the association of sumoylation with the Wnt signaling pathway. PIASy-mediated sumoylation of a member of the Tcf family, LEF-1 inhibits its transcriptional activity while Axam (SENP2)-mediated desumoylation promotes transcription activation (237). Furthermore, PIASy targets LEF1 to the nuclear bodies, suggesting that subnuclear sequestration away from its target genes accounts for the repression of LEF1 activity. Sumoylation of the second component of the pathway, Tcf-4 triggered by PIASy and inhibited by Axam, results in modulation of its β-catenin-dependent transcriptional activity (310). Despite the lack of interaction of Tcf-4 and β-catenin, Tcf-4, SUMO-1 and PIASy are co-localized in the nucleus and associated in the PML body. The nuclear pore complex also contributes to the regulation of the Wnt signaling by sumoylation (259). Immunoprecipitation of TCF-4 has pulled down a number of proteins associated in the nuclear pore complex, including Ran, RanBP2, and RanGAP1. Overexpression of NPC proteins stimulates the nuclear import of the TCF-4 and β-catenin proteins and enhances transcriptional activity. It has been found that NPC proteins enhance, whereas SENP2 suppresses, the growth of colorectal cancer cells.

E. Enhancement of protein stability

Earlier studies have shown that the sumoylation of a substrate increases its protein stability by antagonizing ubiquitination. In some substrates [*e.g.*, IκBα (100), Smad4 (255), Hungtingtin (264), PCNA (218) and promyelocytic leukemia zinc-finger protein (PLZF) (136)], SUMO and ubiquitin are attached on the same lysine residue, with the result that SUMO competes with ubiquitin and antagonizes ubiquitin-dependent proteaosome degradation. Studies of APL-associated PLZF show that PLZF undergoes redox-mediated modification by SUMO-1 and Ub (135, 136), while disruption of the target site for sumoylation and ubiquitination increases protein stability (37, 136). When SUMO-1 and Ub are co-transfected in cells, the level of Ub-conjugated PLZF is lower than that in cells transfected with Ub only.

The underlying molecular mechanism has been found to be mediated by a new family of SUMO-targeted ubiquitin ligases (STUbL) containing a tandem of SUMO interaction motifs (SIM) and RING finger motifs that selectively targets SUMO-conjugates and proteins that contain SUMO-like domains (226, 294a). This is demonstrated by studying the molecular mechanism of arsenic-induced degradation of PML-RARα fusion and leukemia cell differentiation (151, 275). Upon exposure to arsenic, the increased level of SUMO-conjugated PML triggers polyubiquitination of K48 and proteasome degradation via recruitment of RING-domain-containing ubiquitin E3 ligase (RNF4). Cells transfected with nondegradable sumoylation PML-RARα mutant exhibit impaired cell differentiation. However, the role of sumoylation on stability is complicated, as seen from the regulation of hypoxic induced factor 1-α (HIF-1α) (8, 34, 42), essential in normal embryonic development and tumor growth. Interruption of SENP1 decreases the stability of HIF1-α (42) via interaction of sumoylated

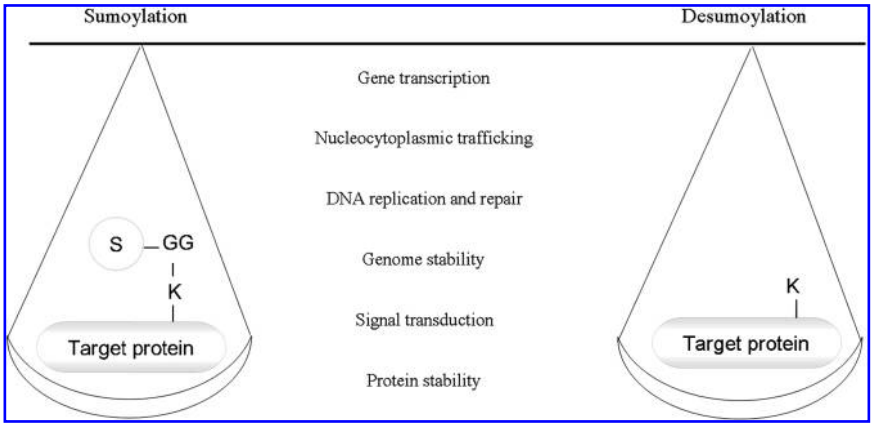

FIG. 9. Biological importance of sumoylation/desumoylation. SUMO modification is involved in diverse cellular events. A proper balance of conjugation and deconjugation of specific target substrates is critical to maintain and regulate the cellular processes upon receiving endogenous and exogenous signals.

HIF1-α with ubiquitin E3 ligase von Hippel-Lindau (VHL). By contrast, in an earlier cell line model SUMO promoted the stability of HIF1-α under hypoxic conditions (8). A hypoxia inducible protein RWD-containing sumoylation enhancer (RSUME) is related to the increased stability of HIF1-α through promoting SUMO-conjugation (34). However, the biological consequence of sumoylation on HIF1-α remains imperfectly understood.

The mechanism by which SUMO modification enhances protein stability in some other substrates, for example APA-1 (15), PPARγ2 (78), Tau (69), SOD1 (75), HIF-1α (42), phosducin (146), PAP (288), and Oct4 (294, 319), also remains unclear.

F. SUMO in cancers

An intensive interplay between SUMO and various essential regulatory cellular proteins hints that SUMO modification is related to carcinogenesis and cancer progression (245). As more oncogenes and tumor suppressor genes are identified as SUMO conjugates (2), the role of SUMO in cancer development seems more obvious and direct (74, 99, 120, 140, 299, 303, 322). It is now believed that alteration of gene expression of components in the sumoylation system is associated to carcinogenesis. This is demonstrated by the upregulation of Ubc9 mRNA level in human ovarian cancer, adenocarcinomas, and prostate cancer (183, 188), upregulation of Ubc9 and SUMO-1 protein levels in response to long-term low-dose radiation in premalignant conditions (234), and overexpression of PIAS3 ligase in breast, prostate, lung, colorectal, and brain cancers (292). A recent *in situ* hybridization study has shown that SENP1 transcript is increased in prostatic intraepithelial neoplasia and prostate cancer lesions as compared with normal prostate epithelia (13, 41), and that the induction of SENP1 is mediated by activated AR.

The roles of sumoylation in cancer metastasis have also been demonstrated by isolating the SENP1- and SENP6-associated β-catenin-reptin chromatin remodeling complex which regulates a metastasis suppressor gene KAI1 (138–140) and inhibits the progression of tumor metastasis (12). Desumoylation of reptin alters its repressive function by inhibiting its association with HDAC1. The SUMO-conjugated reptin level is crucial as it modulates the invasiveness of cancer cells with metastatic potential. It is likely that SUMO is involved in an intricate network with the regulation and participation of various kinds of regulators of cellular processes.

G. SUMO in cardiovascular development

Recent studies have further extended the biological importance of sumoylation to cardiovascular development. A number of transcription factors, including Nkx2.5 (291), GATA4 (289), serum response factor (SRF) (180), and myocardin (290), are SUMO conjugates. Interestingly, these transcription factors all physically interact with each other, suggesting that sumoylation of one transcription factor affects the assembly and stability of the other factors involved in heart development (290). Sumoylation of these transcription factors is enhanced by the PIAS family of E3 ligases. Nkx2.5, a cardiac-specific homeobox gene critical for early heart development and morphogenesis, can be SUMO modified at K51. Sumoylation increases its transcriptional activity through increasing its DNA binding affinity and stabilizing the complex formation of Nkx2.5 with cofactor SRF. Mutation on K51 directs the protein to polyubiquitination, though its subsequent fate remains unclear (291). GATA4, an early expressed gene in heart tissue, functions as a cardiac-enriched transcriptional factor. Sumoylation at K366 upregulates GATA4 transcriptional activation capacity and is also important for its nuclear localization. GATA4 modification is also associated with the initiation of cardiogenic gene activity in fibroblasts (289). Myocardin, containing a SAP (SAF-A/B, Acinus, PIAS) domain, is a coactivator of SRF that promotes SRF-dependent smooth muscle cell differentiation (72). It is also involved in the activation of gene expression of myosin light chain 2 gene (MLC2) with cofactor GATA4 in *Xenopus* (261). SUMO modification of K445 in myocardin serves as a switch to initiate the expression of α-actin and α-myosin heavy chain in pluripotent 10T1/2 fibroblasts which are not activated in normal conditions (293). More specifically, myocardin is targeted by SUMO-1 but not SUMO2/3.

H. SUMO in pathogen–host interaction

Sumoylation of viral proteins, including the early-immediate protein (IEs) of different viruses, affects viral genome replication and virulence (23, 25). Cytomegalovirus (CMV) IEs are potent transcriptional activators localized in the NBs and can be SUMO-modified at K175 and K180. An IE2 double mutant transfection experiment has shown a 5- to 10-fold reduction of transactivation of CMV promoters (110). On the other hand, when CMV IE1 is modified by SUMO, the sumoylation of PML is downregulated (194). In bovine

papillomavirus (BPV) and human papillomavirus (HPV), IE1 mutant defective in SUMO modification loses the ability to accumulate in the nucleus, resulting in a loss of replication capacity (228). Its sumoylation site is located at the region which is crucial for its helicase activity (174), interaction with IE2 and oligomerization (278, 279).

On the other hand, some viral proteins have been reported to influence the sumoylation of PML in the host. The Epstein Barr virus (EBV) Z protein consists of a sumoylation site, and reduces PML sumoylation by competitive inhibition (1). Herpes simplex virus (HSV) ICPO protein also downregulates PML sumoylation, though not by competitive inhibition (220). Intriguingly, PML NB is required for p53 activation and nuclear import (79, 212). Use of nuclear import proteins to bypass the nuclear barrier seems to be a common strategy adopted by IEs of certain viruses to ensure their replication and survival (31). There are also proteins that exist as a common interaction target for SUMO and viral proteins, particularly in HPV, for example tumor suppressor p53 (184), pRb (153) and histone deacetylases (HDACs) (53).

Apart from viral proteins, YopJ protein of *Yersinia pestis* (208), a virulence factor that inhibits the MAPK pathway, exhibits SUMO-specific protease activity that mimics the functions of SENPs. YobJ mutant defective in desumoylation loses its ability to inhibit the MAPK pathway, and its virulence is consequently attenuated.

I. SUMO in other human diseases

1. Palatogenesis. Two recent studies using SUMO-1 knockout mice have demonstrated that SUMO-1 haploinsufficiency due to balanced reciprocal translocation of SUMO-1 gene (46,XX,t(2;8)(q33.1;q24.3)) is associated with unilateral cleft lip and palate (3, 221)., Cleft palates began to develop in ~10% of the heterozygous pups or embryos. Embryonic lethality in the homozygous groups suggests that SUMO-1 is essential in development. Further investigation of the overlapping expression pattern with SUMO-1 and other cleft palate genes in the palatal shelf epithelium and mesenchyme has identified Eya1 as a potential candidate. The occurrence of cleft palates was significantly increased by 36% in compound heterozygotes SUMO1$^{Gt/+}$ Eya1$^{+/-}$ as compared with SUMO-1$^{Gt/+}$ (8.7%) or Eya1$^{+/-}$ (0%). As Eya1 is a substrate for SUMO-1 modification *in vivo*, it is suggested that the sumoylation regulates a network of genes that converge in palate development. Notably, the proteins MSX1, SATB2, and SMAD4, which are related to palate morphogenesis, are also SUMO substrates (65, 77, 154, 168).

2. Type I diabetes. Although the biological function of SUMO-4 is unknown, two independent studies have shown that this unconjugatable SUMO paralogue can modulate the activities of heat shock transcription factors and IκBα and is susceptible to Type I diabetes (27, 94). These observations are supported by the distinct tissue distributions of SUMO-4 in pancreatic islets, immune tissues, and kidney (27, 73, 159). Both insulin-responsive glucose transporter 4 (GLUT4) and negative regulator of insulin receptor protein-tryrosin phosphatase 1B(PTP1B) are SUMO-modified upon insulin stimulation. It is proposed that the conjugation promotes membrane accumulation of GLUT4 and inhibits PTP1B activity that would regulate glucose uptake (51, 84, 150).

VI. Regulation of Sumoylation/Desumoylation

The upregulation of global sumoylation level under conditions of oxidative stress conditions, and the identification of additional new SUMO substrates in yeast and mammalian cells when treated with H_2O_2, imply that the sumoylation pathway is subjected to redox-regulation (57, 103, 163, 173, 178, 215, 287, 324). As sumoylation is a dynamic process, either activation or inhibition of the pathway would have a profound effect on subsequent cellular events. Understanding the regulatory mechanism of sumoylation would help to determine the biological significance of sumoylation and SUMO-mediated signal transmission. In general, regulation of a biological process can be achieved by controlling (a) the transcriptional level of the enzymes or reactants, (b) the activities of the enzymes via translational modification, (c) the interaction of regulators, and (d) the compartmentalization of the enzymes or reactants. Studies using different approaches, including *in vivo*, *in vitro,* and crystallographic studies, have reported that the active sites of SUMO ligases and SUMO proteases are inhibited under oxidative stress (29, 308). Here, we will first discuss recent findings on the posttranslational modification of these enzyme components upon oxidation followed by other types of regulation.

A. Redox regulation of SUMO ligases/proteases

1. Reactive oxygen species and oxidative stress. Of the various stress conditions, oxidative stress is of particular interest due to the diversified nature of its production and cellular effects. Reactive oxygen species (ROS) are composed of diverse chemical species including superoxide anions, hydroxyl radicals, and hydrogen peroxide. It is generally considered that most intracellular ROS production occurs at two independent points in the electron transport chain from the mitochondria, viz. complex I (NADH dehydrogenase) and complex III (ubiquinone–cytochrome *c* reductase), and that the latter is the main site of ROS production (281). Superoxide anion (O^-_2) is the primary metabolite of O_2 by NADPH oxidase (198), while many other ROS are formed via dismutation of superoxide anion. The dismutation of superoxide anion to H_2O_2 is very rapid under normal conditions, and H_2O_2 is freely diffusible and relatively long-lived. In contrast, the highly reactive hydroxyl radical (HO^+) is extremely unstable and requires a metal such as iron during the dismutation reaction (Fig. 10). HO^+ has been measured in a broken cell preparation but is probably not found in intact cells (4, 46). Under the catalysis of superoxide dismutase, dismutation of superoxide anion to $O_2{}^{-2}$ has also been observed (182).

ROS such as O^-_2 and H_2O_2, generated intracellularly by the action of phagocytic ROS-generating enzymes NADPH oxidase, are pumped into the phagocytic vacuole for efficient microbial killing and assume an essentially beneficial role in the host immune system (238). ROS also serve as a second messenger in various receptor signaling pathways. In the early insulin signaling cascade, insulin stimulation is followed by rapid production of H_2O_2 that inhibits PTPs and enhances tyrosine phosphorylation of proteins (175). Insulin-dependent H_2O_2 generation is essential in the regulation of downstream insulin signaling, involving the activation of phosphatidylinositol (PI) 3′-kinase, serine kinase Akt, and ultimately cellular glucose transport in response to insulin (176). Another well-known example is vascular endothelial growth factor (VEGF), which is an endothelial cell-specific growth and

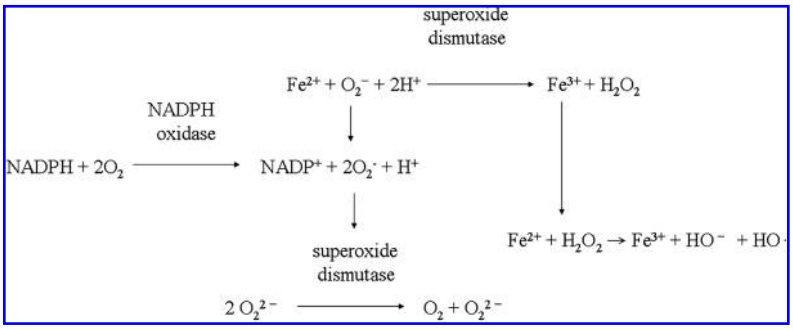

FIG. 10. Production of reactive oxygen species via NADPH oxidase and superoxide dismutase.

survival factor and the major inducer of vasculogenesis and neoangiogenesis (202). VEGF transfers the signals to endothelial cells mainly through two tyrosine kinase receptors, Flt-1 and Flk-1/KDR (214). The activation of KDR by VEGF causes a rapid increase in the intracellular generation of hydrogen peroxide, and inhibition of this kind of ROS generation attenuates early signaling events including receptor autophosphorylation and binding to a phospholipase C-γ-glutathione *S*-transferase fusion protein (47). Some studies have suggested that ROS participate in normal aging and regulate longevity. The best known example is the ROS-resistant forkhead proteins which are highly conserved throughout their evolution, and are able to extend the longevity of worms (92) and mammals (147, 280). As ROS are used in many cellular pathways, a lower level of ROS may interrupt the physiological role of oxidants in cellular proliferation and host defense. Nevertheless, excess ROS, regarded as unwanted by-products of oxidative phosphorylation, lipid metabolism, and ionizing radiations, can overoxidize proteins, resulting in cell damage. Abnormal cellular redox status is a causal factor in a number of diseases, including certain types of cancer, atherosclerosis and neurodegeneration (76).

2. The redox-sensitive cysteine residues. For cell signaling, redox-sensitive cysteine residues serve as transducers at different cellular redox states to allow proteins to respond to ROS. The SH group of cysteine residue can be converted to reversible -SOH, disulfide and cyclic sulfenamide, irreversible SO_2 and SO_3, or even Cys-S-NO, which is formed by further modification of reactive nitrogen species (RNS) (11, 80). A growing body of structural and biochemical data also indicates that cysteine residues are invariably involved both in detecting changes in redox status and in mediating the changes in protein structure and function (56, 112, 241). For instance, the two redox-active cysteines of *E. coli* OxyR transcription factor form an intramolecular disulfide bond when it is oxidized. Such 'fold editing' involves a significant structural change in the regulatory domain of OxyR that brings the two cysteines together from an original 17 Å distance in the reduced form (44). In eukaryotes, 2-Cys peroxiredoxins have been found to be more sensitive to oxidative inactivation than those in prokaryotes, and serve not only as antioxidants but also as regulators of H_2O_2-mediated signal transduction. Structural analysis has shown that this striking feature is conferred by oxidation of the active-site cysteine to a sulfenic acid by the peroxide substrate and recycling to a thiol (300). Another example of post-translational modification of cysteinyl thiols in the presence of oxidants is guanosine 3′5′-monophosphate (cyclic GMP or cGMP)-dependent protein kinase isoform Iα (PKGIα) (30). Oxidation activates PKGIα via the formation of an interprotein disulfide bond that subsequently increases the substrate affinity. The results are consistent with the inducing effect of H_2O_2 on vasorelaxation of the coronary vasculature. In addition to the formation of intermolecular/intramolecular disulfide linkage, a novel mechanism of protein modification has been identified in PTP1B (241). Oxidation of PTP1B induces formation of the sulphenylamide form, generated via covalent interaction of the sulfur atom of catalytic cysteine with the main chain nitrogen of an adjacent residue, which is accompanied by structural changes that inhibit substrate binding. It has been suggested that the cyclic sulfonamide protects PTP1B from irreversible oxidation and allows redox regulation via reversible reduction by thiols.

3. Oxidization effect on SUMO ligases. In sumoylation, cysteine residues are essential in the SUMO conjugation reaction, in which they participate in the formation of E1-SUMO and E2-SUMO thioester intermediates (60, 274). Recent experiments have provided compelling evidence that a low concentration of H_2O_2 (1 m*M*) inhibits SUMO-1 and SUMO-2 conjugation *in vivo* in various mammalian cell lines. This phenomenon has been explained by the rapid and reversible deconjugation and transient accumulation of free SUMO-1 after H_2O_2 treatment for a brief duration. In the case of the three previously identified SUMO substrates, RanGAP1 and AP-1 components c-Fos and c-Jun (28, 181, 193), oxidation has no effect on the sumoylation level of RanGAP1 but reduces those of c-Fos and c-Jun significantly (29). An intermolecular disulfide bond has been observed between Cys173 of Uba2 (the large subunit of E1) and Cys 93 of E2 in both *in vitro* and *in vivo* assays. Intriguingly, the same disulfide linkage of E1-E2 can also be induced by endogenous ROS production, caused by a respiratory burst in macrophages. The disulfide linkage between the two active cysteines can inhibit the catalytic activities of SUMO E1 and E2 ligases, suggesting that ROS is one of the key regulators for the SUMO conjugation process.

4. Oxidizative effect on SUMO-specific proteases

a. Oxidation-induced SENP inhibition. As with SUMO ligases, the active sites of SUMO proteases are also cysteine residues. Our group has characterized the oxidization effects on SUMO proteases (308). The various *in vitro* hydrolytic activities of human SUMO protease SENP1 are inhibited after treatment with 10 m*M* H_2O_2, and the catalytic activities are partially recovered by the reducing agent dithiothreitol (DTT). Formation of the DTT-sensitive disulfide intermolecular dimer of SENP1 *in vitro* was observed. The oxidization of active-site

cysteine, followed by disulfide linkage formation, is responsible for the loss of the enzyme activity. An exposed cysteine residue Cys 613 was further found to be the other major site for dimerization. The full-length SENP1 dimer was also observed and demonstrated in human CHO cells upon exposure to an oxidative stress (as low as 1 m*M* H_2O_2 for 15 min), indicating the physiological implications of the oxidization effect on SUMO proteases. Similar intermolecular interaction was also found in yeast Ulp1 but not human SENP2 *in vitro*. It was also observed in a crystallographic study that three oxidized forms of Ulp1, with the catalytic cysteine oxidized to sulfenic, sulfinic, and sulfonic acids. More importantly, an *in vitro* DTT recovery assay demonstrated that the order of the recovery ability of the three proteases is consistent with the degree of dimer formation. SENP2 with the lowest ability to form a dimer cannot be reactivated by a reducing agent. Collectively, the reversible disulfide linkage of SENP protects the enzymes from irreversible inhibition under oxidizing conditions. However, more *in vivo* experiments are needed to elucidate the biological consequence of the oxidation of SUMO proteases.

From the sequence alignment of various SUMO proteases in humans and yeast, all SUMO proteases (except Ulp2) belong to cysteine proteases. This implies that all known SUMO proteases, excluding Ulp2, may be sensitive to oxidative stress. The protein sequence of Ulp2 suggests that it may be a serine protease and have a higher tolerance to oxidative condition. The SUMO protease SENP5 also contains the corresponding Cys 613 in SENP1, one of the major sites for disulfide linkage formation, suggesting that SENP5 may also employ a similar protective mechanism as that shown in SENP1. However, further research is needed to determine whether other reversible or irreversible enzyme inhibition mechanisms are activated in response to oxidative stress.

A comparison of the overall structures of three oxidized Ulp1 demonstrates that there is no significant structural change, except for some minor rearrangements around the oxidized active cysteine. Notably, the other cysteines in the oxidized forms of Ulp1 are in the reduced form, indicating that the oxidative effect on SUMO proteases is only targeted to the active cysteine. A comparison of the Ulp1 structure with the previously reported Ulp1-SMT3 complex structure reveals that Ulp1 has a similar structural rearrangement at its catalytic triad, which is also found in the SENP2-SUMO-1 complex structure. Efficient cysteine oxidation requires cysteine in a thiolate anion form ($Cys\text{-}S^-$). However, the p*Ka* of the sulfhydryl group of most cysteine residues is 8.5, making it difficult for most cysteines to undergo oxidization. The structure implies that Ulp1 is in an active form without substrate binding. The active-site cysteine C580 is coordinated by the general base His514, which is in turn stabilized by Trp488. This structural arrangement in the catalytic triad confers nucleophilicity on the catalytic cysteine, which therefore has a relatively lower p*Ka*, rendering it in a readily oxidized form. Interestingly, the finding is dissimilar from many other cysteine proteases such as HAUSP, a member of the UBP family of ubiquitin-specific proteases (113), in which substrate binding promotes alignment of the active cysteine to the catalytic histidine for deprotonation. This might explain why only limited structural and functional information on oxidized cysteine proteases has been obtained so far (16).

When taken together, these observations demonstrate that both SUMO proteases and SUMO ligases serve as redox sensors and effectors that undergo reversible and irreversible modification upon exposure to various degrees of oxidative stress (Fig. 11). Since both SUMO conjugation and deconjugation systems are involved in oxidative regulation, the

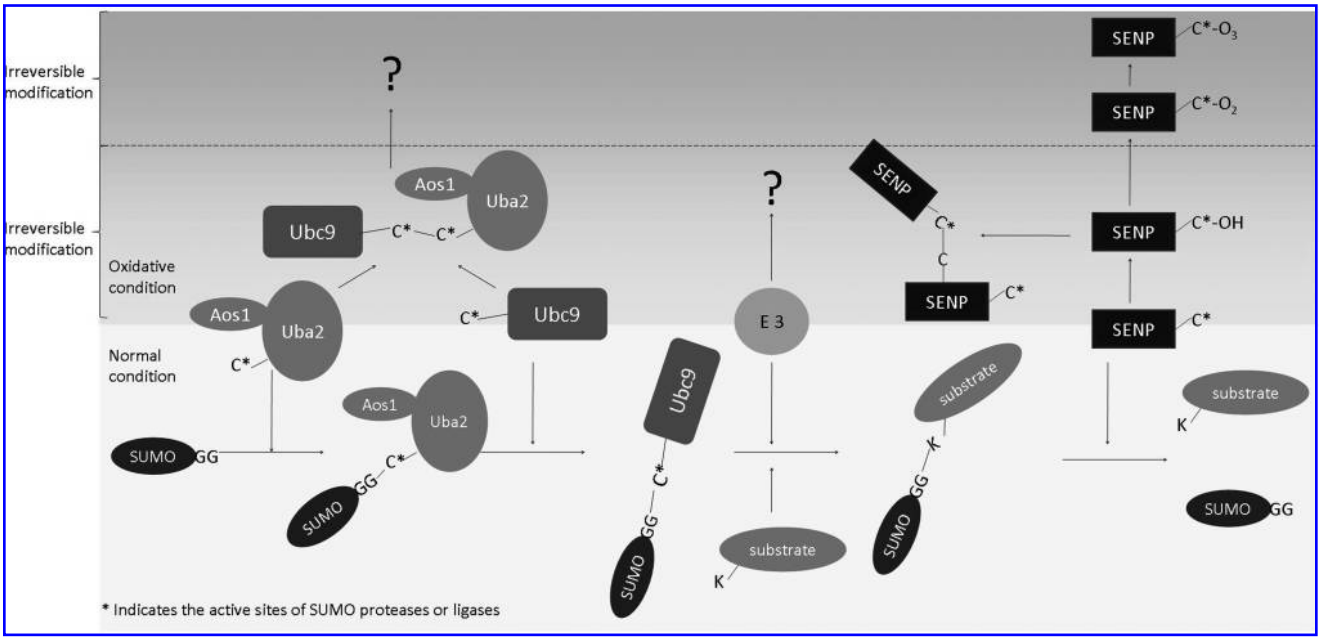

FIG. 11. Redox regulation of sumoylation/desumoylation. In oxidative conditions, the active sites of E1, E2, and SUMO proteases are oxidized to form reversible E1–E2 and SENP–SENP complexes, thus losing their normal enzymatic activities. Their enzymatic activities can be further recovered in the reducing condition by the cleavage of the disulfide bond between the complex. Under more vigorous oxidative conditions, the active cysteine can be further oxidized to irreversible cysteinesulfinic acid and cysteinesulfonic acid, thus completely losing the enzymatic activities.

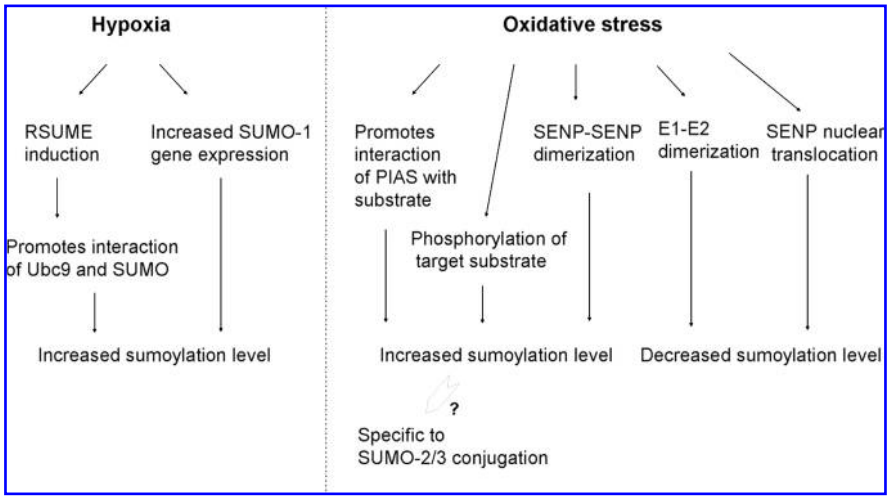

FIG. 12. Integration of current knowledge about the hypoxic effects and oxidative effects on sumoylation/desumoylation. Hypoxia-induced RSUME promotes the interaction of Ubc9 and SUMO, and thereby triggers the SUMO modification of substrates. Upregulated sumoylation level is also reported. This is correlated with the increased transcription level of SUMO-1. On the other hand, the influence of oxidative stress is generally associated with the other post-translational modification. The three routes directing to an increase of sumoylation level are: (1) activation of MAP kinase pathway that enhances the interaction of PIAS and substrate, (2) oxidation-induced phosphorylation of target substrate that triggers its sumoylation via the phosphorylation-dependent sumoylation motif (PDSM), and (3) dimerization of SENPs or irreversible oxidation of SENP that inhibits the desumoylation. Downregulation of sumoylation upon oxidation can be due to the formation of intermolecular E1–E2 disulfide linkage and nuclear translocation of SENP.

redox-regulatory effects on sumoylation system are of some intricacy, and further studies are required to determine precisely how sumoylation and desumoylation are influenced in this process.

b. Oxidation-induced SENP translocation. An earlier study showed that differential intracellular localization of SENP1 is due to the cellular context of different cell lines, and that the export of SENP1 to the cytoplasm is in a nuclear export factor CRM1- and C-terminal NES-dependent manner (144). A recent study of the underlying mechanism of TNF-mediated cytoplasmic translocation of HIPK1 and ASK1-dependent apoptosis has made interesting findings in respect of the redox regulation of SENP1 (165). The study has shown that TNF-induced desumoylation of HIPK1 occurs via the nuclear translocation of SENP1. Intriguingly, SENP1 complexed with thioredoxin (Trx) was detected in the cytoplasm. The translocation requires the dissociation of SENP1 from Trx, and this can be triggered by TNF or 0.5 m*M* H_2O_2, and blocked by *N*-acetyl-cysteine. More importantly, 60 min after TNF-induction, SENP1 is found in the cytoplasm. Notably, the return of SENP1 from nucleus to cytoplasm correlates with its phosphorylation state mediated by the TNF-activated JNK pathway. The findings provide new insights into the redox-regulation of SENPs, though only nucleocytoplasmic shuttling of SENP1 has been reported so far. It seems that cells recruit an alternative mechanism to modulate the desumoylation pathway in addition to the previously described intermolecular disulfide-linked complex formation. Interestingly, Trx has also been shown to interact with apoptosis stimulating

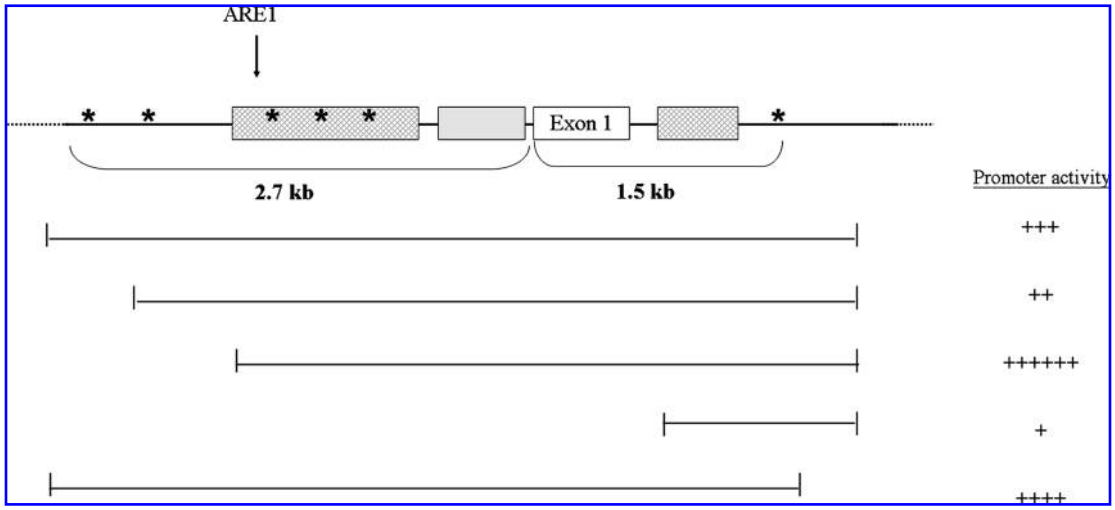

FIG. 13. Schematic illustration of the promoter sequence of SENP1 regulated by activated androgen receptor in prostate cancer cells. Only the 5′ untranslated region of the SENP1 gene is shown. The six potential androgen response elements are indicated by *asterisks*. Two conserved regions and a CpG island are indicated by *hatched* and *filled boxes*, respectively. Translation start site is on exon 2. The results of the promoter assay of different truncated fragments as described by Bawa–Khalfe *et al*. (13) are summarized. Activated androgen receptor directly binds to the ARE1 site.

kinase (ASK1) to regulate the p38 MAPK stress response signaling in a ROS-dependent manner (112). It is not known how these molecules assemble, but it is possible that their interactions might involve cysteine residues. This would then imply that SENP1 is in an inactive form when it is bound on Trx. On the other hand, the importance of NES and phosphorylated SENP1 in the nuclear export needs further investigation. As mentioned in Section III, both SENP1 and SENP2 contain NLS and NES (10, 118), and further studies are required to determine whether the shuttling of SENP2 is also redox regulated. Nevertheless, it would be interesting to identify whether other SENPs also interact with Trx or any other molecules that would modulate their localization and activities upon oxidative stress.

5. Oxidation-induced sumoylation. The sumoylation of some protein substrates under oxidative stress has been investigated. A recent study has shown that PIASy is necessary for NFκB activation by oxidative stress (induced by 2 m*M* H_2O_2) and genotoxic stress. Both stimuli strengthen the interaction of PIASy with NFκB essential modulator (NEMO) and increase the sumoylation of NEMO (115, 173). For MAPK/ERK5, H_2O_2 induces its sumoylation, leading to the downregulation of its transcription activity (257, 258). Interestingly, another study of p53 has demonstrated that the upregulation of sumoylation is specific to the SUMO isoform. Exposure to 5 μM H_2O_2 in HEK293 cells causes an increase of SUMO-2/3 conjugated p53, but not the SUMO-1 conjugated form (164). Similar results were obtained in response to As_2O_3 exposure (103). Another example is highlighted by transcription repression of PLZF (135, 136). A lower intracellular ROS level induced by serum deprivation results in a reduction of SUMO-conjugated PLZF but an increase of Ub-conjugated PLZF, suggesting an antagonistic relationship between sumoylation and ubiquitination upon oxidation. By contrast, it has been reported that sumoylation of most substrates, with the exception of an unusually stable RanGAP1, is lowered in 1 m*M* H_2O_2-treated cells (29). These findings indicate that the oxidative effect on sumoylation may be in a case-dependent manner, and that the effects on the specific SUMO substrates need to be characterized case by case.

6. Hypoxic effect on sumoylation mediated by SENP. The cAMP-response element-binding protein (CREB) offered the first example of the effect of hypoxia on sumoylation (50). In prolonged hypoxia, CREB is modified by SUMO-1, and overexpression of SUMO-1 stabilizes CREB and enhances CREB-dependent reporter gene activity. Yamaguchi *et al.* (309) reported that mice derived from the ES cell line with disruption of the enhancer region on the SENP1 gene exhibited reduced SENP1 expression and abnormality in the development of placenta blood vessels. The biological significance of SENP1 has further been demonstrated by studying its effect on erythropoietin (Epo) production during red blood cell differentiation (42, 302). The expression of the epo gene was under the control of HIF1α in response to oxygen availability. Transgenic mice lacking SENP1 developed severe fetal anemia stemming and died in mid-gestation. It has also been found that sumoylated HIF1α promotes its interaction with ubiquitin ligase, VHL protein and subsequent proteasome-dependent degradation (104, 114, 119). In addition to the degradation mediated by hydroxylation of HIF1α upon hypoxia, SUMO in this circumstance acts as an alternative signal to bind to VHL, thereby activating the transcription of the Epo gene. Accordingly, a model of how SENP1 regulates HIF1α stability in hypoxia has been proposed. VHL mediated degradation of HIF1α is inhibited via blockage of hydroxylation of HIF1α upon hypoxia. On the other hand, hypoxia induces sumoylation of HIF1α in the nucleus and subsequently HIF1α is degraded via an alternative signal for VHL ubiquination. The role of SENP1 is to stabilize HIF1α by removing the alternative VHL-binding signal. Intriguingly, another investigation has shown the identification of a RSUME that promotes sumoylation by noncovalent interaction of SUMO to Ubc9 and enhances Ubc9 thioester formation (34). Sumoylation of HIF1α and IKBα has been shown to be increased upon hypoxia via induction of RSUME. In addition to these specific SUMO substrates, sumoylation has also been shown to augment cell survival against hypoxia-induced injury in rat salivary epithelial cells (203). This finding suggests that hypoxic stress may affect the overall sumoylation profile in cells. The overall hypoxic effects and oxidative effects on sumoylation are illustrated in Fig. 12.

B. Gene expression regulation of SUMO-specific proteases

The desumoylation pathway can be regulated at the transcription level of SENPs. The gene expression of SENP1 is relatively well characterized, and has been shown to be modulated via the AR in prostate cancer cells. In *in situ* hybridization studies, an elevated level of SENP1 mRNA in prostatic intraepithelial neoplasia and prostate cancer cells has been observed in comparison with normal adjacent prostate epithelia (41). Androgen treatment is specific to SENP1, but not to SENP2, though they share over 57% homology in the catalytic domain and are categorized in one independent subfamily. Interestingly, this feature is only observed in prostate carcinoma cells and not in normal prostate epithelial cell line.

The SENP1 gene is located on chromosome 12 and contains a large 5′ prime untranslated region (UTR) with the translation start site on exon 2. Six potential androgen response elements (ARE) are predicted in the 5′ prime UTR, and the activated AR could bind either one or more of the three potential AREs located proximal to the promoter (Fig. 13). Specifically, ARE1 is a high-affinity binding site for the AR. However, it is still unknown what regulates SENP1 mRNA levels in normal prostate epithelial cells. Modulation of SENP1 expression leads to meaningful biological responses, such as changes in AR-mediated cellular proliferation, AR-dependent transcription, and c-Jun-dependent transcription. Furthermore, SENP1 could be an important target for future therapeutic treatments of advanced prostate cancer. It is anticipated that therapeutic agents designed to selectively lower SENP1 levels (40, 42) would be more effective than androgen ablation therapy.

Upregulation of SENP1 mRNA has also been reported in thyroid oncocytic cancer by microarray analysis (121). Seven of the 18 differentially expressed nuclear-encoded genes are involved in protein metabolism. They include DKFZP434I116, B3GTL, SNX19, RP42, SENP1, UBE2D3, and the CTSB gene, which are shown to be deregulated in most thyroid tumors.

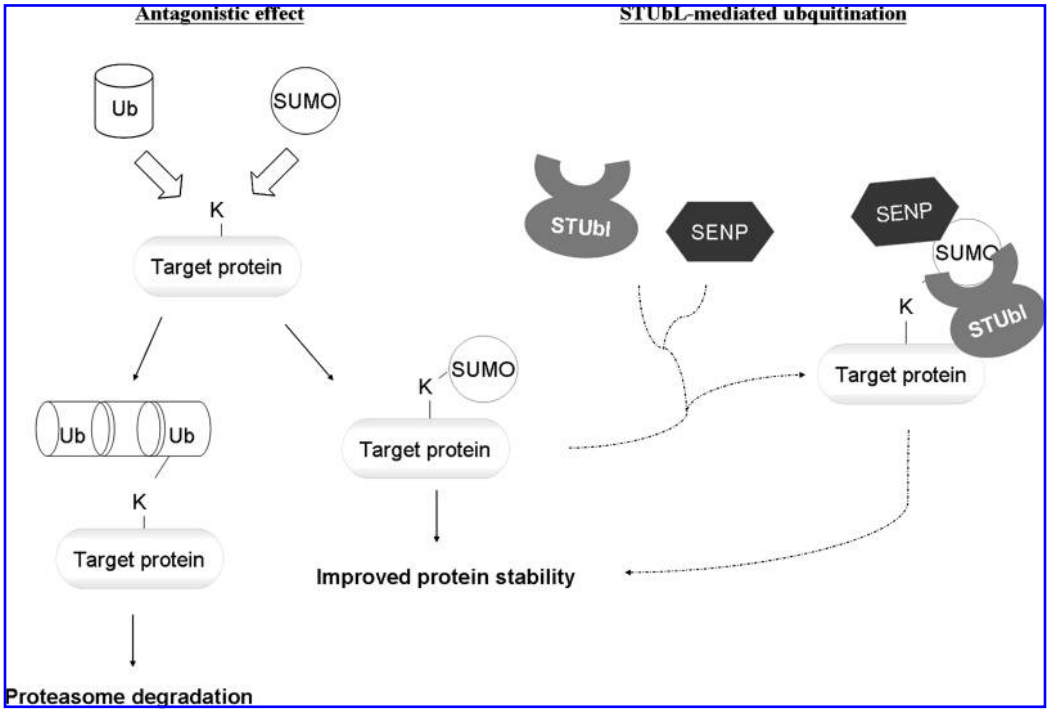

FIG. 14. Interplay of sumoylation and ubiquitination on protein stability. For the antagonistic effect, SUMO and Ub compete with the same target lysine residue on the protein substrate. Polyubquitination of the substrate will direct the protein to proteasome degradation, while sumoylation will enhance protein stability. Alternatively, degradation of protein substrate can also be mediated by SUMO-targeted ubiquitin ligase (STUbL). Recognition of the sumoylated substrate by the tandem SUMO interaction motifs of STUbL triggers desumoylation and ubiquitination concomitantly.

C. Regulators of sumoylation

1. Sumoylation enhancer. A recent study has identified a novel protein co-factor, RSUME, that enhances the overall sumoylation pathway (34). The RWD domain of RSUME shares significant similarity with three major RWD-containing proteins–RING finger-containing proteins, WD-repeat-containing proteins, and yeast DEAD-like helicases—and is named after these three proteins (67). RSUME was firstly cloned from a pituitary lactosomatotrophic tumor cell line with an increased tumorigenic and angiogenic potential (35), and is upregulated by cellular stress stimuli such as hypoxia and heat shock.

RSUME is highly conserved in higher vertebrates. Human RSUME is composed of 195 amino acids, with a single RWD domain spanning residues 3–115. The structure of mouse GCN2 RWD domain reveals a structural homology to mammalian Ubc9, although their protein sequences share only 13% similarity. The core structure appears to be conserved while variations are mainly positioned on the molecular surface, and this may be responsible for a specific protein–protein interaction for different RWE-containing proteins (199). A direct effect on *in vitro* sumoylation is shown by increased SUMO-1 conjugation after addition of recombinant RSUME (34). Despite its structural similarity with Ubc9, the RWD domain lacks a structurally equivalent active cysteine of Ubc9 and does not show any conjugation activity. Further research to determine the crystal structure of the RSUME-Ubc9 complex and interacting proteins may provide insights into these mechanisms.

RSUME is distributed in the nucleus and cytoplasm, and is co-localized with Ubc9. It is expressed in a wide range of tissues and is more abundant in the cerebellum, pituitary, heart, kidney, liver, stomach, pancreas, prostate, and spleen. The functional role of RSUME is closely associated with two central players, NF-κB/IκB and HIF1α, in mammalian angiogenesis and tumorigenesis. RSUME increases IκB levels by promoting its sumoylation through direct interaction with Ubc9 and inhibits NF-κB transcriptional activity. This leads to the inhibition of Cox-2 and IL-8 and the consequent activation of HIF-1α and VEGF, one of its targets for vascularization. NF-κB signaling also plays a role in cancer development and progression, controlling tumor angiogenesis, invasiveness, and resistance to apoptosis (137). On the other hand, RSUME is induced by hypoxia and upregulates the level of sumoylated HIF1α. It should be noted that the stability of HIFα upon sumoylation is still arguable (8, 34, 42). Nevertheless, considering also that hypoxia induces a transcriptional response that is important for both normal embryonic development and the growth of tumors (156), the regulation of HIF1α by RSUME upon hypoxia would be an interesting target for anticancer therapy.

2. SUMO-targeted ubiquitin ligase (STUbL)-mediated homeostasis of sumoylation. The half-lives of sumoylation substrates can be modulated by STUbL (Fig. 14). STUbL contains tandem SUMO interaction motifs at the N-terminus which enhance interactions with SUMO conjugates or proteins that possess SUMO-like domains. STUbL family members are evolutionarily conserved and include Slx6-Slx8 in *S. cerevisiae* (282a, 294a, 304) and Rfp-Slx8 in *S. pombe* (226). The latter STUbL is a heterocomplex with both subunits consisting of RING finger domains, but Slx8 exhibits RING-dependent E3 Ub ligase activity. *S. pombe* mutant *Slx8-Rfp*

shows an accumulation of sumoylated proteins, and displays genomic instability and hypersensitivity to genotoxic stress, suggesting that STUbL is essential for DNA repair and genetic integrity. Human RNF4 having the SIMs and sharing similarity with the C-terminal hydrophobic motifs of the Rfps and RING finger domain with Slx8 has been identified as a STUbL (268). In fact, RNF4 can rescue the phenotypes of *slx8-Rfp* and restore the sumoylation pathway homeostasis. Two SUMO substrates subjected to STUbL-mediated ubiquitination have been identified so far. They are DNA repair protein Rad60 and its human homolog NIP45. A model of STUbL-directed ubiquitination which incorporates the possibility that target proteins are desumoylated and ubiquitinated concomitantly is proposed. When a target protein is subjected to sumoylation, upon completion of its function, it is downregulated via STUbl-dependent desumoylation and degradation simultaneously. In the absence of STUbL, the protein target may be retained in a cycle of sumoylation/desumoylation, resulting in a net increase in sumoylated proteins in the cell. Depending on the process involved, STUbLs may function to clear sumoylated proteins that may otherwise inhibit downstream cellular events.

3. Regulation mediated by viral proteins. Several years ago Colombo *et al*. (49) reported the first study of inhibition of the sumoylation pathway by viral proteins. Adenovirus early gene product Gam1, essential for viral replication (85), can modulate the gene expression in the host cell, via inactivating HDAC1 (43). Expression of Gam1 has been found to destroy PML NB formation, diffusion of SUMO-1 into cytoplasm, and to interfere with the sumoylation of HDAC1 (49). It was later shown that Gam1 specifically interfered with the formation of an E1-SUMO thioester complex (23). A later study demonstrated that the C-terminal SOCS domain of Gam1 interacts with two cellular cullin RING ubiquitin ligases to recruit E1 for ubiquitination and degradation (24, 26). The growing list of SUMO substrates includes many viral proteins, and SUMO appears to facilitate viral infection, making it a potential target for antiviral therapies.

D. Other regulatory mechanisms

Protein molecules can be subjected to more than one post-translational modification. These modifications can occur either in a concomitant or sequential manner. In addition, modification by one modifier can have either synergistic or antagonistic effects on the other. For example, phosphorylation, acetylation, methylation, and ubiquitination of histone H3 and H4 regulate each other in gene transcription (157), phosphorylation of p53 affects its acetylation (22), and acetylation of Smad7 inhibits its ubiquitination (91). Cross-talks between sumoylation and other post-translational modifications have been demonstrated. In particular, associations of sumoylation with phosphorylation and ubiquitination have often been found. Although all current findings are limited to the SUMO ligases and to the conjugation process, determining the underlying mechanism for receiving and transmitting the cellular signal, and coordinating the response with other modifiers would lay a firm foundation for investigating the possible regulatory processes for SUMO proteases in deconjugation. Some representative studies are highlighted in this review to illustrate the linkage between SUMO and other modifiers.

1. Relationship between sumoylation and phosphorylation. Studies have shown that cross-regulation between sumoylation and phosphorylation is exerted via two mechanisms. The first mechanism is to phosphorylate the target substrate that subsequently alters its efficacy to be sumoylated. The second mechanism is to act directly on the enzyme components of sumoylation, that phosphorylation of SUMO ligases or proteases would modulate their activities. The former type was first demonstrated by studying the sumoylation of PML NBs in HeLa cells (196). Treatment of cells with an inhibitor of serine/threonine phosphatases 1 and 2A resulted in a reduction of SUMO-1 conjugated PML. This finding appeared to contradict results showing that phosphorylation of RanGAP1 enhances its sumoylation. It was suggested that phosphorylation, in general, upregulates SUMO-1 modification. Since then, it has been reported that the sumoylation of several other protein substrates, including heat-shock factors (HSFs) (107, 111), GATA-1 (48), and myocyte enhancer factor 2 (89, 133), is subject to a similar type of regulation. Recognition of the close proximity of the sumoylation site and phosphorylation site of these proteins has led to the deduction of a conserved phosphorylation-dependent sumoylation motif (PDSM), ψKxExxSP (108) composed of a SUMO consensus site adjacent to a proline-directed phosphorylation site. Interestingly, PDSM is found in the majority of transcriptional regulators. Furthermore, sumoylation of type I transforming growth factor-beta (TGF-β) receptor, (TβRI) depends on the TβRI activation by phosphorylation, which in turn is induced by the binding of TGF-β to the TβRII–TβRI receptor complex (134). Sumoylation of TβRI promotes the recruitment and phosphorylation of Smad3, which consequently regulates TGF-β-induced transcription and growth inhibition. In contrast, phosphorylation inhibits sumoylation in some cases. Sumoylation of IκBα can be inhibited by its phosphorylation, consequently leading to IκBα degradation and activation of NFκB (58).

For the second mechanism, phosphorylation-induced modification of SUMO ligases, but not that of SUMO proteases, has been reported. Homeodomain interacting protein kinase 2 (HIPK2) is co-localized with SUMO E3 ligase Pc2 and can be modified by SUMO-1 (143, 236). DNA damage induces HIPK2-mediated phosphorylation of Pc2 at multiple sites, and in turn phosphorylation of Pc2 at Thr 495 is required to increase the HIPK2 sumoylation and mediate its transcriptional repression. Interestingly, the interaction between HIPK2 and Pc2 establishes an autoregulatory feedback loop. Another example is provided by the phosphorylation of PIAS1 which plays a critical role in the negative regulation of NFκB/STAT1 signaling (170, 171). In response to various inflammatory stimuli (*e.g.*, TNF, LPS, or IFNγ), PIAS1 is rapidly phosphorylated at Ser 90 through the association of IKKα with PIAS1. Although the S90A variant of PIAS1 retains SUMO E3 ligase activity and interacts with NF-κB, it is defective in inhibiting DNA binding activity of NF-κB and in transcription repression (172). The ligase activity of PIAS1 is necessary for phosphorylation, so that cross-regulation between sumoylation and phosphorylation is essential in controlling the response from pro-inflammatory cytokines and chemokines. Findings on PIASxα-mediated differential responses from transcription factor Elk-1 further highlight the dynamic interplay between the MAPK pathway and the SUMO pathway (311–313). Sumoylation of Elk-1 promotes the recruitment of

HDAC-2 and allows Elk-1 to be maintained in a basal inactivated state. Upon activation of the ERK/MAPK pathway, PIASxα is required to activate Elk-1-regulated immediate-early gene expression (314). However, stress-induced MAPK pathway induces p38-medated phosphorylation of PIASxα and maintains Elk-1 sumoylation, thereby dampening the activation of Elk-1. This finding suggests strongly that PIASxα acts as a key regulator of the sumoylation status of Elk-1. It can act as a signal integrator that, via its phosphorylation, can control the amplitude of Elk-1 activity and target gene expression in response to the different activated MAPK pathways.

2. Relationship between sumoylation and methylation. Previously, we have described an enhancing effect of phosphorylation on sumoylation (*i.e.*, that the phosphorylation of either SUMO ligases or target substrates will trigger sumoylation). The relationship between sumoylation and methylation appears to be different and intricate. Sumoylation of a substrate modulates the methylation level of its associated protein(s). For example, sumoylation of heterochromatin proteins, such as Swi6, Chp2, and Clr4, is required for heterochromatin stability (256). Depletion of SUMO in fission yeast causes an increase of H3 Lys 4 methylation. On the other hand, sumoylation of the methyl-CpG-binding protein MBD1 facilitates its association with MCAF-1, which in turn catalyzes the methylation of histone H3 at Lys 9 (282). Though the molecular mechanism of how sumoylation is linked to histone methylation and heterochromatin formation is unclear, it is possible that the interaction of MCAF-1 and sumoylated MBD1 may stabilize the assembly of methyltransferases at MBD-1 containing heterochromatin regions, or promote the recruitment of chromatin remodeling activity that somehow favors the incorporation of methylated H3. The links between sumoylation and methylation of histone H3 are further exemplified in the transcriptional cofactor KRAB domain-associated protein 1 (KAP1) (156). A complex of KAP1 and its cognate co-repressor KRAB zinc finger protein, ZBRK1 suppresses gene transcription upon doxorubicin (Dox) treatment. The mechanism of Dox-induced p21 expression is shown to be mediated via the sumoylation status of KAP1. In Dox-treated breast cancer cells, the sumoylation level of KAP is transiently reduced. Although the association of KAP1 and ZBRK1 is retained, differential methylation and acetylation of histone H3 at the promoter of p21 is observed. Further research is necessary to establish whether the downregulation of SUMO-conjugated KAP1 is due to the activation of SUMO proteases. A new PcG (Polycomb group) protein, Chromobox4 (cbx4), was identified as a SUMO E3 ligase during investigations of the mechanism by which sumoylation regulates the *de novo* methyltransferase 3a (Dnmt3a), which is responsible for the generation of new methylation pattern during gametogenesis and early development (132, 157, 169). Both *in vitro* and *in vivo* studies have shown that Cbx4 promotes the sumoylation of Dnmt3a and modifies its ability in transcriptional repression.

3. Relationship between sumoylation and acetylation. The integration between sumoylation and acetylation is increasingly linked in promoting transcriptional repression. As mentioned earlier, activation of Elk-1 can be achieved by ERK/MAPK-mediated phosphorylation in response to mitogen stimulation (252). However, as phosphorylation of Elk-1 occurs, there is a rapid loss of SUMO modification of Elk-1. Sumoylation of Elk-1 at the repressor domain results in the recruitment of histone deacetylase activity, in particular that of HDAC2, to promoters (312, 313), leading to a decrease of histone acetylation and hence transcriptional repression of Elk-1 target genes. These findings point to an alternative mechanism in which histone acetylation of Elk-1 regulated promoter can be mediated through MAPK-mediated desumoylation of Elk-1. A recent study of a tumor suppressor, BRCA1, also shows that sumoylation promotes histone deacetylation (219). BRCA1 is associated with familial breast cancers (285, 295) and its interaction with diverse transcriptional regulators suggests that it is also involved in transcription and modulation of chromatin structure. Interaction of SUMO-1 with BRCA1 via the SUMO interaction motif of BRCA1 represses its transactivation potential by inducing the recruitment of HDAC1 activity to the BRCA1-regulated promoters and consequently leading to reduced histone acetylation. In addition, HDAC4 with SUMO E3 ligase activity promotes sumoylation of the transcription factor MEF2 which is important in muscle cell differentiation and apoptosis (317, 321). Interestingly, the sumoylation site on MEF2 is also subject to dynamic acetylation catalyzed by acetyltransferase CBP and NAD+-dependent class III deacetylase, SIRT1. Sumoylation of MEF2 negatively regulates MEF2 transactivation activity. It is generally believed that transcription repression is mediated by histone deacetylation. However, the combined evidence from these studies indicates that sumoylation has a clear and close association with acetylation for modulating gene transcription. Studies of histone post-translational modification in budding yeast further highlight this relationship (200). All four core histones on the chromatin are sumoylated. It appears that histone sumoylation serves as a negative regulator that counteracts the activation of histone modification by acetylation or ubiquitination and represses transcription. Notably, a higher level of sumoylated histone proximal to the telomeres is observed. Whether this is related to telomeric silencing remains to be elucidated. Other examples demonstrating the linkage between sumoylation and acetylation have also been reported from tumor suppressor HIC1, TDG, and PKA1 (156, 189, 263).

VII. Conclusion and Future Perspectives

During the past decade, sumoylation/desumoylation has inevitably been acknowledged as an important protein post-translational modification. A growing body of evidence, much of it with exciting implications, supports the association of sumoylation with numerous cellular events. The depletion of SUMO clearly induces fatal errors, and aberrant regulation of the pathway is associated with a variety of human diseases. Although studies of the biological functions of the deconjugation process are fewer those of the conjugation process, SENPs acting as deconjugating enzymes are necessary for controlling the SUMO modification status in cells. The down-regulation of SENPs in cell lines, budding yeast, and transgenic mice studies confirms that most SENPs are nonredundant and are essential for gene expression, development, and growth. The work done to elucidate the structures of Ulp1 and SENP1/2 and their conjugate complexes has clarified the molecular basis of maturation and deconjugation, and marks an important milestone in the advance of our knowledge in this field. However, many questions remain to be answered. First,

the current structural information is limited to the catalytic domain, whereas the N-terminal fragment which is believed to be responsible for localization and specific substrate recognition is completely unknown. This may be due to the low expression and stability of recombinant full-length SENPs that hinders detailed biochemical characterization. Differential maturation and deconjugation efficacy of SENPs strongly suggests that the recognition of SUMO by SENPs is only a primary requirement for resumption of the catalytic process. Structural hindrance conferred by the target substrates may explain the discrepancy observed. Further studies to resolve the crystal structures of other SENPs and SENPs/conjugated substrate complexes should help to elucidate the atomic details of substrate interaction and cleavage reaction.

Second, while the proteomic era has produced a list of potential SUMO substrates for verification, identification of substrate specificity of each SENP is definitely required. This will be especially important for clarifying the functional roles of individual SENPs and determining whether desumoylation is promoted by co-localization of the substrates with particular SENPs. As sumoylation is a dynamic process and the target substrates often exist in the unconjugated form, a strategy for isolating SENP-specific target substrates may not be straightforward. Nevertheless, previous immunoprecipitation has successfully purified some ribosomal proteins associated with SENP3. Knock-down/out of a particular SENP in cells, followed by quantitative mass spectrometric analysis, is also another possible approach to determine SENP specificity.

Third, an increasing number of studies have demonstrated an association between sumoylation and other post-translational modifications, in particular ubiquitination. The identification of STUbl, which mediates the homeostasis of sumoylation, is intriguing. It serves as an alternative route that modulates the intracellular level of target proteins instead of the level of the unconjugated form via SENPs. Furthermore, considering that lysine residues can undergo additional modifications, such as acetylation and methylation, we may even have to encounter more complex regulatory networks. Understanding the coordination between these modifications under normal conditions and those in response to different cellular stresses will be crucial. This implies that studies of the downstream effects of SENPs should also monitor the interaction with other modifiers along the pathway, if any.

Finally, our current understanding of the regulatory mechanism is only confined to SENP1 and SENP2 under oxidative stress. The formation of the reversible disulfide-linked dimer is a prerequisite for the protection of the enzymes from irreversible oxidation. Although such an SENP1 dimer can be induced by a low concentration of H_2O_2 in the cell line study, further investigation of the proposed regulatory mechanism upon endogenous ROS production is required to demonstrate the physiological relevance. However, the current model may be applied to active tissues, such as myocardium, which have a higher rate of ROS production. Oxidative effects on SUMO E1 and E2 also induce disulfide linkage, but via a heterodimeric interaction. It is interesting to note that the conjugating and deconjugating enzymes exhibit similar regulatory mechanisms. The redox regulation by the reversible disulfide formation is believed to be applicable to other SENPs, as they all contain cysteine residues for catalysis. Based on the experience with SUMO ligases, conjugation can be induced by various exogenous stimuli and conjugation of a particular substrate can be modulated via phosphorylation of the E3 ligase or phosphorylation of the target substrate. Future studies are required to examine whether SENPs undergo additional modification or whether their catalytic properties are influenced by the presence of other modifiers on the target substrates. The latest findings on oxidation-induced nuclear translocation of SENP1 provide an alternative redox regulatory mechanism. A fuller understanding of the interaction of SENP1 with Trx will provide key information on the intracellular distribution of SENPs. Interestingly, nuclear translocation of SENP1 only induces the desumoylation of HIPK1 but not Daxx, which is also a substrate of SENP1. Understanding how SENP1 targets HIPK1 specifically upon translocation will improve our knowledge of protease substrate specificity. In addition, the discovery of RSUME, which promotes the interaction of Ubc9 and SUMO-1 raises the tantalizing possibility that similar regulators might exist for SENPs. Last but not least, modulation of the gene transcription level may be another way of regulating the deconjugation process. Promoter studies of SENPs would help us to determine whether this was the case. Although considerable and very valuable efforts have been made in the past ten years to shed light on this novel post-translational modification, much research still needs to be done before we fully understand the biological roles of SENPs and their implications for human diseases.

Abbreviations

APL, acute promyelocytic leukemia; As_2O_3, arsenic trioxide; AVP1, adenoviral protease-1; BPV, bovine papillomavirus; Cbx4, cGMP, guanosine 3′5′-monophosphate; Chromobox4; CMV, cytomegalovirus; CREB, cAMP-response element-binding protein, Dox, Dnmt3a, *de novo* methyltransferase 3a doxorubicin; DTT, dithiothreitol; DRP1, dynamin related protein; Epo, erythropoietin; GATA4, GATA binding protein 4; Gpx: glutathione peroxidase (GPx)-like enzyme; HDAC, histone deacetylases; HIF-1α, hypoxia inducible factor-1α; HIPK2, homeodomain interacting protein kinase 2; hnRNP, heterogeneous nuclear ribonucleoproteins; HPV, human papillomavirus; IE, early-immediate protein; KAP1, KRAB domain-associated protein 1; MAPK, mitogen activated protein kinase; MLC2, myosin light chain 2 gene; MRX, Mre11–Rad50–Xrs2; NBs, nuclear bodies; ND10, nuclear domain 10; NEMO, NFκB essential modulator; NES, nuclear export signal; Nkx2.5, NK2 transcription factor related, locus 5; NLS, nuclear localization signal; NPM1, nucleophosmin; Pc2, polycomb group protein; PCNA, proliferating cell nuclear antigen; PKGIα, dependent protein kinase isoform Iα PIAS, protein inhibitor of activated STAT; PLZF, promyelocytic leukemia zinc-finger protein; PML, promyelocytic leukemia; PODs, PML oncogenic domains; PTP1B, protein tyrosine phosphatase 1B; RanBP2, nuclear pore complex (NPC)-associated protein; RanGAP1, Ran GTPase activating protein; RNF4, RING-domain-containing ubiquitin E3 ligase; RNS, reactive nitrogen species; ROS, reactive oxygen species; RSUME, RWD-containing sumoylation enhancer; SAF-A and –B, scaffold attachment factors A and B; SBM, SUMO binding motif; SENP, SUMO specific protease; SIM, SUMO interaction motifs; Smc, structural-maintenance-of-chromosome proteins; SMT3, suppressor of mif two 3; STUbL, SUMO-targeted ubiquitin ligase; SRF, serum response factor; SUMO, small

ubiquitin modifier; TβRI, type I transforming growth factor-beta (TGF-β) receptor; Trx, thioredoxin; TDG, thymine DNA glycosylase; TopII, topoisomerase II; Ub, ubiquitin; Ubl, ubiquitin-like proteins; VHL, von Hippel-Lindau;

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Address reprint requests to:
Shannon W. N. Au
Centre of Protein Science and Crystallography
Department of Biochemistry
Faculty of Science
The Chinese University of Hong Kong
Hong Kong

E-mail: Shannon-au@cuhk.edu.hk

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