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Cellular and Molecular Life Sciences

Review

Sumoylation regulates diverse biological processes

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Abstract. Ten years after its discovery, the small ubiquitin-like protein modifier (SUMO) has emerged as a key regulator of proteins. While early studies indicated that sumoylation takes place mainly in the nucleus, an increasing number of non-nuclear substrates have recently been identified, suggesting a wider stage for sumoylation in the cell. Unlike ubiquitylation, which primarily targets a substrate for degradation, sumoylation regulates a substrate's functions mainly by altering the intracellular localization, protein-protein interactions or other types of post-translational modifications. These changes in turn affect gene expression, genomic and chromosomal stability and integrity, and signal transduction. Sumoylation is counter-balanced by desumoylation, and well-balanced sumoylation is essential for normal cellular behaviors. Loss of the balance has been associated with a number of diseases. This paper reviews recent progress in the study of SUMO pathways, substrates, and cellular functions and highlights important findings that have accelerated advances in this study field and link sumoylation to human diseases.

Keywords. SUMO, sumoylation cycle, desumoylation, gene expression, genomic and chromosomal integrity, signal transduction, loss-of-function of SUMO pathway, SUMO-associated disease.

Introduction

SUMO nomenclature

To date, four mammalian SUMO proteins have been identified, including SUMO-1 (also known as PIC1, UBL1, sentrin, GMP1 and SMT3C), SUMO-2 (also known as SMT3A), SUMO-3 (also known as SMT3B), and SUMO-4. Although none of the SUMOs was discovered with the aim of finding new ubiquitin-like proteins [1], SUMO – for small ubiquitin-like protein modifier – has become the widely accepted term. This is not only because this term unifies the SUMO family and descriptively ties the family to ubiquitin, but also because it is pronounced exactly as the popular Japanese-style wrestling.

SUMO discoveries

Whereas the cloning of the human SUMO-3 by EST screening and of the mouse SUMO-2 and SUMO-3 by genomic PCR screening [2, 3] did not initially provide clues about where and how the proteins might function, the cloning processes *per se* of the mammalian SUMO-1 and human SUMO-2 and SUMO-4 have clearly indicated their primary subcellular locations, potential functions, and disease relevance. For example, yeast two-hybrid screening identified human SUMO-1 as a binding partner of the nuclear body protein PML [4], the DNA repair proteins RAD51/RAD52 [5], and the cytoplasmic death domains of the cell surface death receptors Fas and tumor necrosis factor (TNF) receptor 1 [6], suggesting nuclear roles for SUMO-1 in the regulation of gene expression and genomic integrity as well as a non-nuclear role

associated with apoptotic processes. In addition, peptide sequencing identification of rat SUMO-1 as a binding partner of the nuclear pore complex protein RanGAP1 suggested an important role of SUMO-1 in the nucleocytoplasmic shuttling of proteins [7, 8]. Furthermore, human SUMO-2 was discovered by cDNA selection directly from the telomeric region of chromosome 21q that is associated with Down syndrome [9]. Finally, single-nucleotide-polymorphism screening led to the identification of human SUMO-4 as a candidate protein that is strongly associated with susceptibility to type 1 diabetes [10, 11]. These findings highlight the functional significance of SUMOs in cellular functions and human diseases.

SUMO pathway versus ubiquitin pathway: similarities and differences

Similarities

SUMO and ubiquitin share a similar protein size, tertiary structure, and a C-terminal di-glycine motif. The sumoylation cycle (Fig. 1) is also remarkably similar to that of ubiquitylation. Both SUMO and ubiquitin proteins are synthesized as precursors. The immature precursors are first processed by the specific C-terminal hydrolase, which removes the C-terminal tail so that the di-glycine motif becomes available for activation and conjugation. The mature SUMO or ubiquitin then starts a process involving their ATP-dependent activation by the E1 enzyme, conjugation by the E2 enzyme, and binding to their substrate with help from the E3 ligase. Upon the completion of the process, SUMO or ubiquitin can be recycled by deconjugating-enzyme-catalyzed dissociation from the substrate.

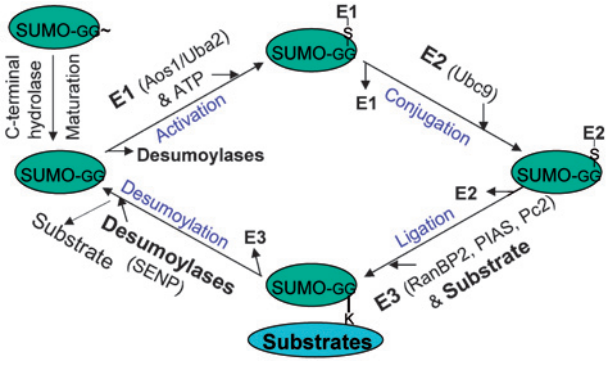

Figure 1. SUMO pathways. C-terminal-specific hydrolase-catalyzed removal of C-terminal tail from SUMO precursor renders the di-glycine motif of the matured SUMO available for E1-catalyzed SUMO activation, E2-catalyzed SUMO conjugation, and E3-mediated SUMO ligation to the substrate proteins. Desumoylation counter-balances sumoylation by freeing the substrates from SUMO binding.

Differences

The most visible and well-established role of ubiquitylation is to target the substrate proteins for proteasomal degradation. However, the primary function of SUMO appears to be to modify the substrate activity or function rather than protein stability. The ubiquitin pathway has only one form of ubiquitin, with two single-subunit E1 s [12], a large number of E2 s, and several hundred substrate-specific E3 s, whereas the SUMO pathway uses more than one SUMO, a heterodimeric E1, a single E2, and just a few E3 s with rather broader substrate specificity. Furthermore, while ubiquitin E3 s are present everywhere in the cell, the SUMO E3 s as well as desumoylases seem to be localized to specific subcellular compartments. For example, the mammalian SUMO E3 protein inhibitors of activated STAT (PIAS) proteins and SENP1 desumoylases are localized to the nucleoplasm and the nuclear bodies, RanBP2 (E3) and SENP2 to the nuclear pore, the polycomb group protein Pc2 (E3) to the polycomb body, SENP3 in the nucleolus, and SENP6 (or SUSP1) in the cytoplasm [13–18]. The distinct subcellular localization is believed to contribute to a large part of SUMO substrate specificity.

SUMO-pathway-dependent biological functions: insights learned from loss-of-function studies

Genetic studies have linked the SUMO pathway proteins to many critical functions at both cellular and organismic levels. The primary findings are summarized below [For a complete recent account see ref. 19].

SUMO

SUMO-1 haploinsufficiency, or disruption of the SUMO-1 locus due to a balanced reciprocal translocation has been associated with cleft lip and palate in a human patient [20]. A causative role for SUMO-1 in the development of lip and palate was supported by studies using SUMO-1 knockout mice. Cleft palate developed in almost 10 % of the heterozygous pups or embryos but not in the wild-type mice. The embryonic lethality or immediate postnatal death of the knockout mice pointed to the essential developmental functions of SUMO-1 [20]. Studies in lower species including *Caenorhabditis elegans*, *Schistosaccharomyces pombe* (fission yeast), and *Saccharomyces cerevisiae* (budding yeast) also suggested an essential role of SUMO-1 in cell-growth- and mitosis-associated events such as centromere and kinetochore formation, chromosome segregation, and chromatid separation. Additionally, a polymorphismic mutant variant of SUMO-4 (at nucleotide A163G resulting in M55V

mutation) has been associated with human type 1 diabetes [10, 11].

E1

Both AOS1 (also known as SAE1) and UBA2 (also known as SAE2) of SUMO E1 subunits are essential for the G2-to-M transition of the cell cycle in budding yeast [21, 22], although, interestingly, deletion of Aos1 (rad31) merely leads to DNA damage sensitivity in fission yeast [23]. Ablation of UBA2 leads to embryonic lethality in *C. elegans* [24].

E2

The SUMO E2 enzyme Ubc9 plays an essential role in early embryonic development and this role is evolutionally conserved. Ubc9 knockout mouse embryos die at the early postimplantation stage. Ubc9-deficient cells derived from the knockout embryos show severe defects in nuclear organization, including nuclear envelope dysmorphy, disruption of nucleoli, and PML nuclear bodies (NBs), defects in chromosome condensation and segregation, and failure of RanGAP1 to accumulate at the nuclear pore [25]. Loss of Ubc9 function also leads to embryonic lethality in *C. elegans*, G2-to-M phase arrest in *S. cerevisiae*, and meiotic defects in *Drosophila melanogaster* (fruit fly). Interestingly, depletion of Ubc9 in the chicken lymphoma cell line DT-40 leads to cytokinesis defects without errors in chromosome condensation and segregation [26]. Whether this apparent discrepancy reflects a difference between embryonic cells and a transformed cell line or between species is a question that merits further study.

E3

Unlike fruit fly and *C. elegans* in which only one form of the SP-RING (Siz/PIAS RING [27]) family E3 was identified (*Su(var)2–10* or *zimp* and *gei-17,* respectively), more than one such E3 has been discovered in fission yeast (*nse2* and *pli1*), budding yeast (*SIZ1*, *SIZ2*/*NFI1* and *MMS21*), and mammals (PIAS1, PIAS3, the α and β spliced forms of PIASx, and PIASy encoded by four genes). Loss of PIAS function leads to embryonic lethality in both the fruit fly and *C. elegans*, with abnormal body morphology, and the PIAS-deficient cells show defects in chromosome segregation and telomere assembly. Surprisingly, knockout of PIASx or PIAXy does not result in any significant defects in the mouse development or in the SUMO-associated cellular functions, suggesting that the E3 s could be either dispensable or redundant [28–30]. Deletion of *nse2* and *MMS21* is lethal to fission yeast and budding yeast, respectively [31–33], whereas *pli3* and the *SIZ* E3 s are not essential. Although *pli*-deleted fission yeast cells display no obvious mitotic growth defect, these cells are sensitive to the microtubule-destabilizing drug TBZ and exhibit deregulated homologous recombination and marked defects in chromosome segregation and centromeric silencing [34]. The mutant cells also show a consistent increase in telomere length due to increased telomerase activity resulting from impaired sumoylation in the cells [35]. Neither of the *SIZ* proteins is essential for budding yeast viability, and the mutant cells with deletion of both *SIZ* genes remain viable, although such deletion removes $>90\,\%$ of the SUMO conjugates in the cells [36]. Interestingly, while deletion of *MMS21* is lethal, cells harboring the *MMS21* E3 catalytically inactive mutant are viable [33]. These results suggest that the E3 activity of *MMS21*, which may be responsible for the $<10\,\%$ sumoylation in the cells, is not required for cell viability, and neither is the E3 activity of the *SIZ* proteins. Indeed, the *SIZ* double-deletion mutant grows poorly at low temperature and such defects can be rescued by deletion of yeast 2 μm plasmid [37]. The *SIZ*-depleted mutant cells also exhibit defects in minimicrosome segregation which are attributed to the lack of sumoylation on topoisomerase II [38]. There seems to be a large overlap between the substrates of *SIZ1* and *SIZ2* [39]. Notably, however, differences exist between the two *SIZ* E3 s. For example, the *SIZ2* exclusively localizes in the nucleus, whereas *SIZ1* functions in the nucleus or the bud-neck depending upon the mitotic cycle [40]. Whether this differential localization confers the highly *SIZ1* (but not *SIZ2*) dependent cytotoxicity by doxorubicin [41] is an interesting question to address.

Desumoylase

There are at least seven desumoylases known as SENP (SUMO/sentrin-specific protease) encoded by six genes in mammalian cells [For more information, see ref. 42]. Loss of SENP1 function leads to embryonic lethality in mice, presumably owing to placental abnormality [43]. Constitutional t(12;15)(q13;q25) chromosomal translocation between SENP1 and MESDC2 (an endoplasmic reticulum protein related to embryonic polarity) that disrupts both genes has been associated with an infantile sacrococcygeal teratoma of a human patient [44]. In addition, it has been found that SENP6 is fused to TCBA1 (T cell lymphoma breakpoint associated target 1) to form a SENP6-TCBA1 chimerical gene at chromosome band 6q21 (one of the most frequent target regions in T cell lymphoma) in the human T cell lymphoma cell line, HT-1 [45].

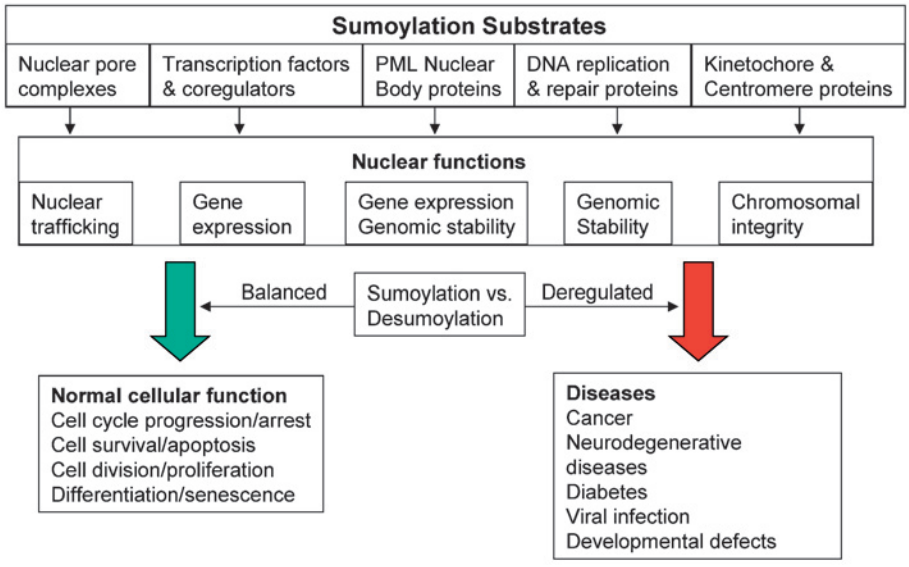

Figure 2. Sumoylation substrates and their functions. The majority of the substrates are nuclear and participate in the indicated nuclear functions. A regulated balance between sumoylation and desumoylation is essential for normal cell behaviors, and loss of the balance leads to diseased states.

Substrates and biological outcomes of sumoylation

More than 120 mammalian substrate proteins have been identified so far (Table 1). A recent proteomics study has predicted many more additions to the rapidly growing list [46]. The vast majority of the substrates belong to the nuclear proteins, highlighting the primary nuclear functions of sumoylation (Fig. 2). However, growing numbers of non-nuclear or even foreign proteins have been identified, suggesting that some important non-nuclear roles of sumoylation have been underestimated. Nevertheless, more than two-thirds of the known substrate proteins have at least one consensus sumoylation motif ψKxE/D (where ψ is a large hydrophobic residue such as Val, Ile, Leu, Met, or Phe and x is any residue), and between one-third to a half of human proteins share this motif [46, 47]. These findings suggest a potentially much larger pool of sumoylation targets in the cell. [For exact positions of the sumoylation motif on some of the substrate proteins documented, see refs. 46, 48, 49].

Transcription regulators

The primary nuclear substrates are transcription factors and co-regulators (Table 1). In most cases, sumoylation either enhances the function of the transcription repressors or co-repressors, or inhibits the function of the transcription activators or co-activators. In some cases, sumoylation even turns a transcription activator into a repressor [48, 50–53]. Although this suggests that the prominent effect of sumoylation on transcription is repression, growing lists of transcription repressors that are inhibited by sumoylation and transcription activators that are activated by sumoylation indicate a more complicated role of sumoylation in the regulation of transcription.

PML NB-associated proteins

NBs are interface subnuclear punctate structures also known as ND10 (for nuclear domain 10), PML (for promyelocytic leukemia) bodies or POD (for promyelocytic oncogenic domain) [54]. PML, the NB-associated tumor suppressor phosphoprotein, was initially identified in patients with acute promyelocytic leukemia (APL) where it is fused to the retinoic acid receptor α (RARα) gene as a result of the t(15;17) chromosomal translocation [55]. This APL-causing gene fusion disrupts NBs in the nucleus. Sumoylation of PML is required for NB formation and recruitment of other NB-associated proteins including Sp100 and transcription regulators such as Daxx, HDAC1, CBP, p53, Sp3, and LEF1. Depletion of PML results in loss of NBs that can be rescued by re-expression of wild-type PML but not sumoylation-deficient PML mutants [56, 57]. Besides PML, the other proteins are also found sumoylated in the NB, suggesting that sumoylation modulates their interaction in the NB. Their sumoylation likely takes place in the NB given the fact that sumoylation-deficient mutants of most of the proteins still localize to the NB, and all the PIAS E3 s have been found to co-localize with the NB [52]. The exact function of NB remains obscure. It could serve as either a nuclear storage or a specific active site of the associated proteins. Nevertheless, since transcription regulators are the main components in the NB, their sumoylation and interaction in the NB must play an important role in the regulation of transcription. Indeed, disruption of the NBs by the early gene products of various DNA

Table 1. Effects of sumoylation on substrate proteins.

Transcription factors and co-factors
Transcription activators Inhibited – AP-2, AR, ARNT, BMAL1, C/EBP, c-Jun, c-Myb, Elf4, ELK-1, ERM, Ets1, GATA-1, HsTAF5, IRF-1, Lef1, MEF2A, MEF2C, MEF2D, NF-IL6beta, P63, P73, PR, PLAG1/2, PPARgamma2, PRB, RXRalpha, Smad4, Sox3, SOX6, SOX10, Sp1, Sp3, SREBPs, SRF Activated – APA-1, CREB, ER, FAT1, HIF1α, HSF, MITF, P45/NF-E2, p53, Tcf-4 Activated or inhibited – GR Transcription repressors Activated – Huntingtin, MafG, MEF2, PLZF, RBP1, Sam68, SnoN Inhibited – LIN1, KAP1, MBD1, P66, SIP, TEL, ZNF76 Not sure – Msx1 Dual transcriptional activator/repressor proteins Inhibited/activated – Tr2, Net, Reptin Inhibited/inhibited – KLF8 Transcription co-activators Inhibited – AIB1, CBP, GRIP1, MKL1, p300, Sox2 Transcription co-repressors Activated – CtBP1, HDAC1, HDAC4, N-CoR Inhibited – Dnmt3a Histone silencers Inhibited – MBD1 Histone proteins Switch transcription from activation to repression – all four nucleosomal core histones (2A, 2B, 3 and 4)
PML nuclear body proteins Promote nuclear body formation – PML, Sp100 Promote nuclear-body-associated transcription – CBP, Daxx, HIPK2, hRIPbeta, P53, TEL, ZNF198
DNA replication/recombination/repair (R1/R2/R3) associated BLM – R3↑, Rad52 – R2↑, TDG – R3↑, TOP1 – R3↑, TOP2 – R3↑, WRN – R1/R2↑, XRCC4 – R2/R3↑, PCNA – R3↓
Kinetochore and centromere complex associated Cenp-C – centromere cohesion↓ and sister chromatid separation↑, Pds5p – chromosome cohesion↓, RanGAP1 – microtubule-kinetochore assembly↑, TOP2 – chromosome cohesion/segregation at centromeres↑
Other nuclear proteins ADAR1 – RNA editing↓, MDM2 – stabilization↑ and p53 degradation↑, preribosomes – formation and nuclear export↑
Nuclear pore complex targeting RanGAP1↑
Cytoplasmic proteins APP – stabilisation and aggregation↑, atrophin-1 – stabilisation and aggregation↑, Axin – JNK activation↑, CamKII – ?, caspase-7 – nuclear targeting?, caspase-8 – nuclear targeting?, dMek1 – stabilization and nuclear export↑, DRP1 – mitochondrial targeting↑ and stablilization↑, dynamin – endocytosis↓, FAK – autoactivation↑ and nuclear targeting?, Glut1 – destabilization↑, Glut4 – stabilization↑ and cytoplasmic trafficking?, HIPK2 – phosphorylation and activation of Pc2↑, hNinein – centrosome-to-nucleus trafficking↑, IκBα- stabilization↑, NEMO – NFκB modulation↑, PDGFc – nuclear translocation?, phosducin – stabilization↑, PP2C – ?, procaspase-2 – nuclear targeting? and nuclear body targeting↑, PTP1B – catalytic activity↓, SOD1 – stabilization and aggregation↑, Tau – stabilization and aggregation↑, Tax – NFkB activation↑
Transmembrane proteins Fas – death induction↓, K2P1 – potassium ion transport↓, mGluR8 – G receptor signaling?, TNFR1 – death induction↓
Viral proteins AV5 E1B – viral transforming ability↑, AV Gam1 – SUMO E1 stability↓, activity↓ and overall host sumoylation↓, CAV apoptin – PML NB targeting↑, DV2E – plaque formation↓, EBV Rta – viral lytic activity↑, HCMV IE1 – PML sumoylation↓ and viral yield/growth↑, HCMV IE2 – replication site targeting↑, HHV-6 IE1 – ?, KSHV K-bZIP – repressor activity↑, MMLV CA – viral replication↑, PV E1 – intranuclear accumulation↑ and replication↑, SARS-CoV N – host cell division↓, VV A40R – viral replication site targeting↑ and self-association↓

↑, ↓ or ? indicates positive, negative or unclear effect by sumoylation of the indicated substrate, respectively.

viruses such as the adenoviral Gam-1 protein, which inhibits SUMO E1, results in the inhibition of the NB-associated proteins and profound changes in their regulated transcription [58].

Proteins associated with DNA recombination, replication and repair

The genome in the cell is constantly damaged by extrinsic and intrinsic factors. To survive, eukaryotic

organisms have evolved highly conserved DNA damage repair mechanisms to ensure that the genome is copied faithfully during each cycle of cell division. Most repair jobs are done before the S phase by mechanisms such as base excision repair. Occasionally, however, some lesions can sneak into S phase and cause stalled or broken replication forks, possibly giving rise to more serious lesions. In this case, the cell uses an alternative mechanism known as postreplication repair to remove or bypass the lesions. The SUMO E3 ligase Mms21/Nse2 catalyzes the sumoylation of the Smc5/6 complex that participates in the repair of double-strand breaks; consistently, disruption of the ligase function leads to increased sensitivity to DNA damage [31–33, 59].

The most intriguing example is the sumoylation of the proliferating cell nuclear antigen (PCNA) in the postreplication repair process [for reviews see refs. 60, 61]. PCNA serves as a sliding processivity clamp for replicative DNA polymerases and plays a key role in DNA replication and repair. Ubiquitylation of PCNA at lysine 164 takes place in a manner that does not direct PCNA for degradation (mono-ubiquitylation or poly-ubiquitylation at K63, but not K48 of ubiquitin). Instead, such a modification is required for both error-prone (when mono-ubiquitinated) and error-free (if poly-ubiquitinated) postreplication repairs. Recent studies have demonstrated that sumoylation of PCNA prevents the error-free repair by recruiting the anti-recombinogenic DNA helicase Srs2 to the replication forks [62, 63]. This seemingly controversial cross-talk between ubiquitylation and sumoylation of PCNA suggests that modification of PCNA is critically fine-tuned and that the cross-talk appears to ensure the completion of postreplication repairs without yielding abortive recombination events.

Finally, the base excision repair enzyme thymidine DNA glycosylase (TDG) catalzes the removal of the aberrant U or T from the G:U or G:T mismatch lesions. The TDG must be then released from the apurinic (G:_) site for the downstream enzymes to restore G:C pairs. Sumoylation of TDG has been shown to help with this release by reducing TDG binding affinity to DNA [64–66].

Proteins associated with chromosome assembly and segregation

To perfectly copy genetic materials to the daughter cells during cell division depends on precisely orchestrated chromosome dynamics including sister chromatid cohesion, chromosome condensation, and segregation. Earlier studies have demonstrated the role of SUMO pathway components in chromosome dynamics. For example, the budding yeast SUMO (SMT3) and desumoylase (SMT4) were initially identified as high-copy suppressors of the centromere-binding protein Mif2p/Cenp-C [67]. Consistently, SMT3 was later identified as a chromosome cohesion defect gene [31]. Similarly, disruption of the SUMO E2, E3 (*SIZ1* or *Mms21p*), or desumoylase results in spindle defects in fruit flies, chromosome segregation defects in mice, and chromosome segregation, condensation or telomere defects in budding yeasts [25, 28, 33, 68, 69]. In addition, mutation of fission yeast SUMO or E3 (Pli1p) leads to rapid telomere elongation, aberrant mitosis and high sensitivity to microtubule-destabilizing agents [34, 35, 70]. Among known substrates of sumoylation that are involved in these regulations are Cenp-C, topoisomerase II (top2), the cohesion protein Pds5 and nuclear pore complex protein RanGAP1. Recent studies have confirmed that Cenp-C is a target of SUMO1 and this protein plays a key role at centromeres for mitotic progression in human cell lines [71, 72]. Desumoylation of Top2p has been shown to play an active role in maintaining centromere cohesion in budding yeast, suggesting that its sumoylation inhibits the cohesion [73]. Similarly, desumoylation of Pds5 appears to be required for cohesion maintenance, whereas its sumoylation peaks at anaphase and seems to be necessary for dissolution of cohesion during mitosis in budding yeast [74]. Finally, sumoylated RanGAP1 is targeted to the microtubule spindle and kinetochores to guide their attachment during mitosis in HeLa cells [75, 76]. Taken together, it seems that sumoylation promotes chromosome separation whereas desumoylation helps with cohesion.

Other nuclear proteins

Three special nuclear targets have recently been identified. One of them is ADAR1 (adenosine deaminase that acts on RNA), an RNA-editing enzyme. ADAR1 co-localizes with SUMO-1 in a subnucleolar region. When modified by SUMO-1 on K418, its RNA-editing ability is reduced while the sumoylation-deficient mutant promotes the editing [77]. The second is the ribosomal precursor particle. These particles are initially assembled in the nucleolus prior to their transfer to the nucleoplasm and export to the cytoplasm. A recent study in yeast demonstrated that all the SUMO pathway components including SUMO, E1, E2, and even the nuclear pore desumoylase (i.e., Ulp1) are required for the export process and that many ribosome biogenesis factors are sumoylated [78]. This finding suggests that sumoylation of pre-ribosomal particles in the nucleus and subsequent desumoylation at the nuclear pore complex (NPC) is necessary for efficient ribosome biogenesis and export. Lastly, desumoylation of Mdm2, a major ubiq-

uitin E3 ligase that promotes p53 ubiquitylation and degradation has recently been reported to result in Mdm2 self-ubiquitylation and degradation allowing for p53 stabilization [79].

Cytoplasmic and trans-membrane substrates

Although most SUMO substrates are nuclear proteins, ironically, RanGAP1, the first SUMO substrate to be identified, is in fact a cytoplasmic protein that is localized on the cytoplasmic fibrils of the NPC. The centromere-associated role of sumoylated RanGAP1, as described above, is thought to be its minor function. Its major function is actually to activate the small GTPase protein Ran, the key player in the NPC, which governs the nucleocytoplasmic trafficking of proteins. Sumoylation is clearly required for the NPC targeting of RanGAP1 in mammalian cells [7, 8, 80, 81]. Other initially identified SUMO substrates are the transmembrane death receptors Fas and TNFR1 whose sumoylation inhibits their apoptotic signaling [6].

A growing number of non-nuclear substrates have been identified, suggesting that SUMOs can regulate events beyond the nucleus. Most of these proteins are signal transduction proteins. Sumoylation changes the activity, stability, or subcellular distribution of these proteins to eventually alter the signaling events. For example, sumoylation activates FAK (a cytoplasmic protein tyrosine kinase) [82] and inhibits PTP1B (a cytoplasmic phosphatase) [83] and K2P1 (a plasma membrane K^+ channel pore component that promotes K^+ leak) [84]. Sumoylation protects IκBα (the NFκB inhibitor) [85], phosducin (a trimeric G-protein Gβγ subunit-binding and -regulating protein) [86], DRP1 (a mitochondrial-fission-associated dynamin-related protein) [87], Glut4 (a glucose transporter) [88], *Dictyostelium* Mek1 [89], and SOD1 [90] from degradation by ubiquitylation or unknown mechanisms. Sumoylation promotes cytoplasmic redistribution of Glut4 (cytoplasm to plasma membrane) [91], DRP1 (cytoplasm to mitochondria) [87], Mek1 (nuclear export) [89], and the centrosome protein hNinein (centrosome to nucleus) [92]. Finally, it has been proposed that sumoylation may target cytoplasmic or even membrane substrates such as FAK, caspase-7 and -8, and Fas to the nucleus [6, 82, 93]. Taken together, these findings clearly indicate a broader stage of sumoylation in the cell than previously thought.

Viral proteins

Many viral proteins have been identified as sumoylation substrates, and sumoylation seems to facilitate viral infection of the host cells. During infection, the viral proteins somehow inhibit sumoylation of endogenous proteins in the host cells. Several models have been proposed to interpret the underlying mechanisms. For example, the viral proteins could inhibit the SUMO activation or conjugation process, prevent the cellular proteins from accessing SUMO molecules, or promote desumoylation of the cellular substrates. Another possibility is that the viral proteins need to be activated by sumoylation and thus compete with cellular proteins for using the cellular SUMO pathways. As a typical example, adenoviral infection leads to the inactivation of SUMO-activating enzyme E1 by the viral protein Gam1. Gam1 mediates the E1 degradation via recruiting cullin RING ubiquitin ligases, resulting in a global abrogation of sumoylation in the host cells [58, 94, 95, for most recent reviews see refs. 94, 96]. These results provide further understanding of the mechanisms of viral infection and suggest that manipulating SUMO pathways could help antiviral therapies.

Collectively, as outlined in Figure 2, timely sumoylation of cellular protein substrates at the correct cellular compartment would ultimately alter a diverse array of cellular responses including cell cycle progression, survival, apoptosis, division, proliferation, differentiation and senescence. Therefore it is apparent that constitutive deregulation of the physiological dynamics of sumoylation in the cells can eventually lead to diseases (see below).

How does sumoylation change the substrate proteins?

The exact molecular mechanisms by which sumoylation impacts substrate function remain unsolved. In my opinion, it is all about a change in the protein interaction with its binding partners that serve as its modifiers (e.g., ubiquitin, histone acetyl transferases and histone deacetylases, protein kinases and phosphatases) or its traffic carriers (e.g., NPC proteins) (Fig. 3). Such a change may result from SUMO occupation of the identical binding sites for other modifying proteins. This possibility can be tested by comparing protein-interacting profiles of an unsumoylated with a sumoylated form of the same substrate protein. Altered protein interactions eventually change the protein functions either directly, through a change in the expression levels of the protein, or by targeting the protein to a specific subcellular location where the protein does its job or hibernates. Desumoylation does the opposite. Currently known example proteins that are regulated by the mechanisms illustrated in the model are described below.

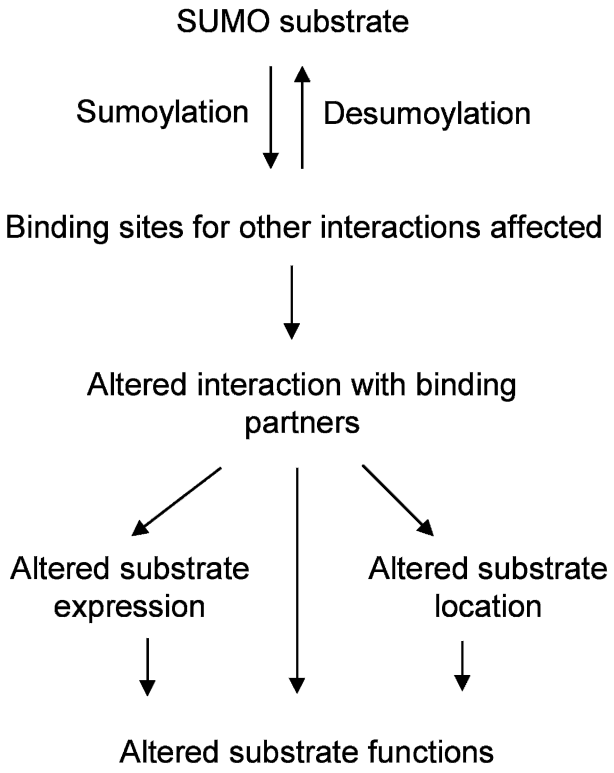

Figure 3. Potential mechanisms for SUMO regulation of its substrate function. Sumoylation may affect interaction of substrate protein with other binding partners of the substrate, resulting in a change in the substrate functions either directly, via modulating stability of the substrate protein, or by targeting the substrate protein to its functional sites or storage foci. Desumoylation plays an opposite role in the process.

Antagonizing ubiquitylation

IκBα [85], Smad4 [97], Huntingtin [98], PCNA [60–62], Rad52 [99], phosducin [86], APA-1 [100], HIF-1α [101], PPARγ2 [102], Tau [103], SOD1 [90] and Mdm2 [79, 104] are all examples of substrates whose degradation by ubiquitylation is protected by sumoylation. The first four proteins fall into the same subgroup in that they all have a single lysine residue that is targeted by both sumoylation and ubiquitylation. In these cases, it is believed that sumoylation and ubiquitylation compete to either prevent or promote proteasome-mediated degradation of the protein. An exception is PCNA. As described in the previous section, ubiquitylation of PCNA at lysine 164 does not target PCNA for degradation, but co-operates with sumoylation at the same lysine maintaining the dynamic interaction between PCNA and Srs2 helicase. This ensures the best quality of postreplication repairs. How sumoylation prevents APA-1, HIF-1α, PPARγ2, Tau and SOD1 from ubiquitin-mediated degradation is not clear. It is clear, however, that sumoylation shelters Rad52 and phosducin from ubiquitin-mediated degradation by modifying lysine residues that are not ubiquitin-binding sites given that their sumoylation-deficient mutants equally suffer from the degradation. Notably, sumoylation of Mdm2 has recently been revisited by two groups. One of the studies demonstrated that SUSP4, a SUMO-specific protease, competes with p53 for binding to Mdm2 and hence removes SUMO-1 from sumoylated Mdm2 resulting in Mdm2 self-ubiquitylation and degradation and eventual p53 stabilization [79]. The other study demonstrated that low levels of Mdm2 catalyze mono-ubiquitylation of p53 to expose the C-terminal nuclear export signal (NES) and to promote sumoylation, resulting in nuclear export of p53 [104]. In either of the cases, the nuclear functions of p53 and hence its diverse cellular functions such as cell cycle progression will be inhibited. These studies reveal the biological significance of the interplays between ubiquitin and SUMO modification in cell signaling and cell cycle control [for recent reviews, see refs. 105, 106].

Preventing acetylation or promoting deacetylation

Acetylation by acetyltransferase co-activators such as p300 and CBP plays a critical role in the activation of gene promoters through their interaction with transcriptional activators. Conversely, deacetylation by deacetylases such as CtBP promotes transcriptional repression. The transcriptional factors MEF2 [107, 108], PLAG1/PLAGL2 [109], NF-IL6β [110] and ELK-1 [111], and the nucleosomal core histones [112, 113] are all cross-regulated by sumoylation and acetylation or deacetylation. MEF2 turns out to be a very interesting case. Sumoylation of MEF2 inhibits its transactivator function, since SUMO modifies the same lysine that is the acetylation site of the transcription coactivator CBP. Interestingly, the sumoylation is facilitated by the HDAC co-repressors. Desumoylation by SENP3 rescues the transactivator function of MEF2 presumably by recruiting CBP to the same lysine residue. Even more interestingly, the sumoylation is also facilitated by phosphorylation of an adjacent serine residue (see below). PLAG1 and PLAGL2 are oncogenic transcription repressors, and it has been shown that sumoylation promotes their repressor function and transforming capability. A sumoylation-deficient mutant of PLAGL2 is less acetylated by the p300 co-activator, suggesting that sumoylation and acetylation share the target lysines. Similarly, NF-IL6β recruits p300 and thus transactivates the Cox-2 promoter upon EGF stimulation, and when sumoylated or fused to SUMO, NF-IL6β no longer binds to p300 and loses transactivator function. ELK-1 is an ERK MAP kinase effector that normally acts as a transcriptional activator by recruiting the p300 co-activator to target gene promoters. When

sumoylated, however, ELK-1 recruits HDAC-2 co-repressor (instead of p300) to the same promoters and thus represses them. It has been shown that ERK activation somehow prevents the sumoylation of ELK-1. Two recent studies have identified histone sumoylation as the first negative regulatory mechanism of transcription in budding yeast [112, 113]. All four histones are sumoylation targets and possess a large number of apparent sumoylation sites (albeit lacking the core consensus motif ψKxE/D [114]). Since the lysines within many of the sites bear modifications for both sumoylation and acetylation, direct competition between these two modifications appears to be one of the mechanisms involved in the repressive role of histone sumoylation [112]. A SUMO-H4 fusion associates with endogenous HDAC1 as well as HP1 (a key structural protein of heterochromatin), suggesting a second repressive mechanism by which histone sumoylation may lead to recruitment of deacetylases [113]. Finally, the repression of genes representing diverse regulatory pathways suggests a quite general repressive role of histone sumoylation [112].

Co-ordinating with phosphorylation

Phosphorylation is one of the widely used post-translational modifications in the cell to regulate protein functions, with IκBα [85], AIB1 [115], MEF2C/D [116, 117], HSF1 [118], and PPARγ2 [119] being examples. As described above, ubiquitylation on K21 of IκBα targets it for rapid degradation whereas sumoylation on the same site prevents the degradation. In fact, the ubiquitylation requires phosphorylation of S32 and S36. This phosphorylation inhibits the sumoylation. Similarly, phosphorylation inhibits the sumoylation of AIB1, the steroid receptor co-activator. In contrast, phosphorylation of MEF2C at S396 or MEF2D at S444 facilitates their sumoylation at K391 or K439; phosphorylation at S303 of HSF1 is required for its sumoylation at K298; and phosphorylation at S112 of PPARγ2 promotes its sumoylation at K107. These results suggest that, like ubiquitylation, sumoylation may occur on many substrate proteins in a phosphorylation-dependent manner. Indeed, closer observation has identified a phospho-sumoyl switch, or PDSM (phosphorylation-dependent sumoylation motif, ψKxExxS/T) that is well conserved in many SUMO target proteins [49, 120]. Another extended SUMO consensus motif from the core motif ψKxE, termed NDSM (negatively charged amino-acid-dependent sumoylation motif), has recently been identified [121]. The NDSM encompasses several acidic residues clustered within the ten-amino-acid region located immediately downstream of the core motif. While the core motif ψKxE interacts with the active site on Ubc9, the acidic tail makes contact with the basic patch on the surface of Ubc9. This 'double-contact' model is very similar to what takes place in the MAP kinase-substrate interaction and is believed to play an important role in determining the efficiency as well as specificity of substrate sumoylation. Interestingly, the phosphate groups attached to the PDSM on MEF2 upon phosphorylation actually bring in negative charges similar to those on the acidic residues, suggesting that it is the negative charges that matter. Notably, together with the PDSM, the NDSM seems to become the most powerful tool for predicting *bona fide* target proteins and modification sites for sumoylation. Several studies have shown that SUMO paralogues can promote non-covalent binding to proteins containing a SUMO-interacting motif (SIM) that consists of a hydrophobic core and a stretch of negatively charged acidic amino acids or a phosphorylated serine [122–124]. Of interest is that the negatively charged stretch determines the binding specificity to distinct SUMO paralogues [122]. Of note, a recently developed Ubc9 fusion-directed sumoylation (UFDS) system seems to strongly enhance substrate-specific sumoylation that usually takes place at very low levels, providing an easy way to test protein sumoylation and to perform more detailed functional analyses [125]. Taken together, the identification of the PDSM, NDSM, and SIM, and the development of the UFDS system make possible faster and more accurate prediction and analysis of SUMO modification and interacting targets.

Nuclear import

The small GTPase protein Ran is a key player at the nuclear pore that transports proteins into the nucleus [126]. The compartmentalized distribution of the RanGAP1 associated with RanBP2 at the cytoplasmic side and the RanGEF RCC1 at the nuclear side of the nuclear pore channel maintains a high enrichment of RanGTP in the nucleus and RanGDP in the cytoplasm. Importins bind to their cargo in the cytoplasm and release the load upon RanGTP binding in the nucleus. The importin-RanGTP complex recycles to the cytoplasm where GTP hydrolysis terminates the cycle. The free importin is then able to repeat the process. Only sumoylated RanGAP1 binds to RanBP2 on the cytoplasmic side of the nuclear pore [7, 81], suggesting that sumoylation is critical for nuclear import of proteins. In support of this, unlike most SUMO substrates, only a small portion is sumoylated, whereas more than 50% of the RanGAP1 pool is sumoylated. RanBP2 has been proposed to act as a SUMO E3 ligase for RanGAP1. However, most of the experiments suggesting this link

were performed *in vitro*. The *bona fide* SUMO ligase *in vivo* for RanGAP1 therefore formally remains an open question. Localization of all the SUMO pathway components including SUMO, Uba2, Ubc9, SENP2, Ulp1 on both cytoplasmic and nuclear sides of the NPC further supports an important role of sumoylation for nuclear transport [15, 47, 127–129]. This also supports a notion that dynamic sumoylation of NPC proteins may have a crucial role in the control of the nuclear import of proteins. Indeed, disruption of either Uba2 or Ulp1 in budding yeast prevents the importin-α subunit Srp1 from recycling to the cytoplasm from the nucleus. This in turn blocks the cNLS-dependent nuclear import, although Srp1 *per se* does not seem to be a SUMO target [130]. It appears that during nuclear transport, the protein is sumoylated in a nuclear localization signal (NLS)-dependent manner as demonstrated for several *bona fide* substrates such as Sp100 and PML as well as artificial targets [47, 131–133]. It is conceivable that these targets are sumoylated at the NPC during the transport. It is also possible that the NLS is required to target these proteins to some subnuclear foci where sumoylation takes place. To distinguish these two possibilities, mutant substrate proteins must be designed such that they remain able to be sumoylated and to target the NPC but fail to pass through the nuclear pore channel and to enter the nucleus. It has also been shown that the sumoylated forms of some other cytoplasmic proteins are localized in the nucleus. Examples include the focal adhesion protein FAK [82], the mitochondrial proteins caspase-7, caspase-8, and procaspase-2 [93, 134, 135], and the centrosome-associated protein ninein [92]. Although it is not clear whether these proteins are sumoylated in the cytoplasm, at the NPC, or in the nucleus, it is conceivable that these proteins exert distinct functions in the nucleus in a sumoylation-dependent manner. Another interesting example is the co-repressor CtBP [136]. In this case, it appears that sumoylation is required to keep it in the nucleus given that its sumoylation-deficient mutant localizes in the cytoplasm. This indicates that the dynamic regulation of the nucleo-cytoplasmic shuttling by sumoylation and desumoylation quantitatively control the nuclear function of CtBP.

Subnuclear targeting

Sumoylated proteins in the nucleus are not usually distributed uniformly. Instead, they localize in many cases as a protein group at distinct individual subnuclear locations. One typical example is the PML NBs (see above). Other examples include PcG (polycomb group) bodies, DNA damage foci, Cajal bodies (CBs), centrosomes, and centromeres. PcG bodies consist of polycomb complex proteins including the Pc2 E3 ligase and some SUMO substrates such as ring finger proteins, co-repressors and chromatin-remodeling factors [137]. It is believed that polycomb complexes induce post-translational modification of histone tails. Such modification leads to induction of a heterochromatin-like state of genes and thus gene silencing. It is likely that sumoylation plays a role in PcG body assembly to influence gene transcription. It is proposed that sumoylation brings DNA repair proteins to DNA damage foci to ensure genomic integrity during DNA replication [138]. Similarly, sumoylated proteins concentrate at centrosomes or centromeres to participate in precise chromosome segregation that is essential for maintaining the chromosome integrity during mitosis [139].

Nuclear export

Sumoylation also helps send nuclear proteins to the cytoplasm. In contrast to CtBP described above, the primary functional site of *Dictyostelium* MEK1 (dMEK1) is in the cytoplasm where it is required for aggregation and chemotaxis. Interestingly, the cytoplasmic localization of dMEK1 depends on its sumoylation, and its non-sumoylated form is predominantly present in the nucleus [89]. Chemoattractant stimulation induces rapid sumoylation of dMEK1, its translocation from the nucleus to the cytosol and the leading edge of migrating cells. Disruption of the sumoylation site on MEK1 prevents its nuclear export, and this mutant cannot rescue the dMEK1-null phenotypes, suggesting that sumoylation is required for both the nuclear export and activation of dMEK1. Another example is the potential tumor suppressor TEL, a transcription repressor. A recent study demonstrates that leptomycin-B-sensitive nuclear export of TEL depends on its sumoylation at residue K99 [140]. Sumoylation has also been shown to promote the transport of preribosomes from the nucleolus to the cytoplasm and it seems that dynamic sumoylation as well as desumoylation is required for this whole process [78]. Notably, Mdm2 has been recently linked to sumoylation-dependent p53 nuclear export [104]. This study demonstrated that Mdm2 at low levels catalyzes mono-ubiquitylation of p53 to expose the C-terminal NES and to promote sumoylation, resulting in nuclear export of p53. This result added another mechanism for restriction of p53 functions by Mdm2.

Subcytoplasmic and plasma membrane targeting

As in the nucleus, sumoylation also targets substrate proteins to specific locations within the cytoplasm. In one example, sumoylation of DRP1, a GTPase protein required for mitochondrial fission, promotes its re-

cruitment from the cytosol to the mitochondrial outer membrane. SUMO-1 specifically protects DRP1 from degradation, resulting in a more stable, active pool of DRP1 at the site of membrane scission [87]. In a second example, a recent study [83] shows that sumoylation inhibits PTP1B activity by confining it to the perinuclear region. Although the molecular mechanisms are not clear, insulin signaling promotes the sumoylation and inactivation of PTP1B. Conversely, the sumoylation-deficient mutant of PTP1B shows more potent activity in the dephosphorylation of insulin receptors. It would be interesting to test whether this mutant is enriched to the cytoplasmic face of the plasma membrane. Finally, the glucose transporter GLUT4 predominantly localizes to cytoplasmic tubulovesicular clusters in close proximity to the plasma membrane. Extracellular insulin signals for rapid translocation of GLUT4 from the cytoplasmic store to the membrane surface results in fast glucose uptake into the cell [141]. Sumoylation has been demonstrated to enhance GLUT4 stability although it is not known whether such a regulation is through sumoylation-facilitated cell surface targeting of GLUT4 [88, 91].

SUMO and diseases

Rapidly growing evidence has been linking SUMO pathways and sumoylation to human diseases. These diseases include cancer, neurodegenerative diseases such as Alzeimer's, Parkinson's, familial amyotrophic sclerosis (FALS) and Huntington's disease, diabetes, and the developmental disease cleft lips with or without cleft palate (CLP). The evidence results from either deregulated expression or chromosomal locations (in most cases through chromosomal translocations) of SUMO pathway machineries or altered functions of sumoylation substrate proteins. Although the causative relationships between the deregulation and pathogeneses of the diseases and underlying molecular basis need extensive investigations, studies so far (see below) have provided strong suggestions that SUMO pathway molecules or SUMO target proteins could eventually be targeted for therapeutic intervention.

Cancer

Overexpression of SUMO-2 and the Uba2 E1 subunit has been correlated with poor survival of hepatocellular carcinoma patients [142]. The Ubc9 E2 has been found overexpressed in human lung adenocarcinomas and ovarian carcinomas [143, 144]. Overexpression of PIAS3 E3 is also reported in several types of human cancer including breast, prostate, lung, colorectal, and brain tumors [145]. These findings suggest a promoting role of sumoylation in human cancer. Interestingly, the SENP1 protease has also been found upregulated in human cancer such as prostate [42] and thyroid oncocytic tumors [146], and transgenic expression of SENP1 in mouse prostate epithelium results in early neoplastic lesions in the prostate [42]. In addition, SENP1-MESDC2 (embryonic polarity-related mesoderm development gene 2) fusions owing to chromosomal translocation at t(12;15)(q13;q25) have been identified from a human patient with infantile teratoma [44]. Similarly, a SENP6-TCBA1 (T cell lymphoma breakpoint associated target 1) chimerical gene has been discovered in a human T cell lymphoblastic lymphoma cell line HT-1 [45]. These results suggest that contribution of sumoylation versus desumoylation to cancer may not be oversimplified. Cancer-associated chromosomal translocations also happen to sumoylation substrates. A typical case is the tumor suppressor PML. It is well established that sumoylation of PML is required for the assembly of PML NBs. In APL cells of human patients, NB formation is disrupted as a result of t(15;17) chromosomal translocations resulting in the PML-RARα (the retinoic acid receptor α) gene fusion [55]. The recently identified potential tumor suppressor TEL, a transcriptional repressor, can inhibit Ras-dependent transformation. TEL is frequently disrupted by chromosomal translocations such as the one at t(12;21), which is associated with nearly one-fourth of pediatric B cell acute lymphoblastic leukemia. A recent report demonstrates that TEL is actively exported from the nucleus in a leptomycin-B-sensitive manner and the export depends on sumoylation at K99, suggesting that the putative tumor suppressor function of TEL in the nucleus is negatively regulated by sumoylation and nuclear export [140].

In addition to these deregulations in gene expression and locations, many proto-oncogenic and tumor suppressor proteins are sumoylation targets. Among the proto-oncogene targets are Bcl2, c-Myb, c-Jun, c-Fos, and PLAG1/PLAGL2 that play a key role in the regulation of general cell proliferation and survival. Other oncogenic signaling pathways regulated by sumoylation include Wnt, NFκB, nuclear receptor transcription factors and their co-regulators. SUMO also controls the activity of key tumor suppressors such as p53, pRB, p63, and p73, as well as Mdm2.

Sumoylation plays an important role in the progression of cell differentiation. During Ca^{2+}-induced differentiation of the human keratinocyte cell line HaCaT, the sumoylation pathway components, including SAE1, SAE2, Ubc9, SENP1, Miz-1 (PIASx-beta), SUMO2, and SUMO3 are highly overexpressed

and activated. Abrogation of sumoylation by Gam1 expression severely disrupts the cell differentiation [147].

Overexpression of PIASy E3 in normal human fibroblasts induces senescence arrest by sumoylation-dependent activation of p53 transcriptional activity and repression of E2F-responsive genes dependent on pRB, and the senescence response in PIASy-null mouse embryo fibroblasts is highly reduced, suggesting that PIASy-mediated sumoylation actively contributes to the execution of the senescence program and hence tumor suppression [148].

Recent studies also link sumoylation to tumor metastasis. In one example, the chromatin-remodeling protein reptin helps recruit the co-activator Tip60 to facilitate the transcription of the tumor metastasis suppressor KAI1. When sumoylated, reptin loses this function and instead facilitates β-catenin-mediated repression of the KAI1 promoter [149]. The integrin signaling mediator FAK plays a critical role in tumor invasion and metastasis. We and others have recently shown that both FAK and its downstream transcription factor KLF8 are regulated by sumoylation [82, 150]. These results suggest that sumoylation may also affect tumor metastasis by regulating this important signaling pathway.

As genome and chromosome instabilities make a critical contribution to malignant transformation and tumor progression, sumoylation of proteins associated with the stability and integrity of the genome and chromosomes would certainly play a part in cancer initiation and progression.

For a more detailed discussion about the role of sumoylation in cancer, the reader is referred to some recent reviews [42, 105, 151–158].

Neurodegenerative diseases

These diseases are protein aggregation disorders characterized by abnormal accumulation in the intracellular inclusion bodies of ubiquitylated misfolded proteins that are otherwise degraded in the proteasomes. The accumulated proteins are toxic to neurons. Among these diseases are Alzheimer's, Parkinson's, and Huntington's diseases, spinal and bulbar muscular atrophy, prion disease, polyglutamine diseases, multiple-system atrophy [159, 160], and amyotrophic lateral sclerosis. Several of the disease proteins are sumoylation substrates, including Tau [103], α-synuclein [103], amyloid precursor protein [161], Huntingtin [98], atrophin-1 [162], androgen receptor [163], and SOD1 [90]. Although causative links of the sumoylation of these proteins to the diseases are not yet conclusive, it appears that the sumoylation stabilizes the proteins, prevents the proteins from ubiquitin-mediated degradation, and promotes aggregate formation in the inclusions. Indeed, SUMO-1 colocalizes with these proteins in the aggregates. Therefore, it appears that in most cases, sumoylation enhances the protein toxicity and promotes neuronal death.

Diabetes

SUMO-4 has been recently cloned in an attempt to identify genes susceptible to human type 1 diabetes mellitus (T1DM) [10, 11]. In these studies, a single-nucleotide polymorphism (A163G) resulting in a substitution of methionine with valine at 55 (M55V) disrupts a putative PKC phosphorylation site (54SVK56). This mutation was strongly correlated with the susceptibility to T1DM, especially in Asian patients and those of European descent in the USA [164, 165]. Another study has linked the M55V mutant to the nephropathy associated with type 2 diabetes [166]. SUMO-4 seems to play a role in sumoylating and stabilizing IκBα leading to inactivation of NFκB. By contrast, the M55V mutant loses this function resulting in overactivation of NFκB signaling. In addition to NFκB, other substrates of SUMO-4 were identified including AP-1, STAT, and HSF family proteins as well as many anti-stress proteins. All these proteins are implicated in autoimmune diseases such as diabetes [167]. Consistent with the above results, SUMO-4 expression is primarily restricted in pancreatic islets, immune tissues and kidneys [10, 164]. Further extensive investigation into these SUMO-4 target proteins is expected to lead to better understanding of the mechanisms underlying the role of SUMO-4 in the pathogenesis of diabetes. Furthermore, proteins that regulate glucose levels in the blood are also regulated by sumoylation. Extracellular insulin interacts with its receptors on the cell surface. This interaction signals the recruitment of the GLUT4 glucose transporter to the membrane from the cytoplasm. The membrane GLUT4 then takes in the glucose, leading to a decrease in glucose levels in the blood. On the other hand, PTP1B dephosphorylates insulin receptors to negatively regulate the insulin receptor signaling. Both GLUT4 and PTP1B are sumoylated in response to insulin stimulation. Sumoylation promotes the membrane accumulation of GLUT4, presumably by enhancing the protein stability and facilitating its trafficking [88, 91]. Interestingly, sumoylation inhibits PTP1B activity and expression [83]. Taken together, these results suggest that sumoylation prevents diabetes by positively regulating insulin receptor signaling.

Viral infection

It is believed that sumoylation of viral proteins in host cells facilitates viral infection, making SUMO path-

ways possible therapeutic targets. Potential underlying mechanisms have been discussed above.

Developmental defects

SUMO-1 haploinsufficiency, or disruption of the SUMO-1 locus owing to a balanced reciprocal translocation, has been associated with cleft lip and palate in a human patient [20]. The same study further confirmed the causative role for SUMO-1 in the development of lip and palate in a SUMO-1 knockout mouse model. Several major signaling pathways including the Wnt3/Wnt9, the BMP2/BMP4 (bone morphogenetic proteins), the FGF8, and the Shh pathways have been found to play critical roles for the development of lip and palate [168]. It will be interesting to find out whether the function of these pathways depends on SUMO-1 and to identify the critical SUMO-1 targets within these pathways during embryonic development of lip and palate.

Perspectives

Over the past ten years, SUMOs have been established as essential regulators of many cellular functions. Aberrant SUMO regulation is a likely cause of a variety of human diseases. Whereas new SUMO targets are identified rapidly, many fundamental questions remain unanswered. What types of cell signaling control the expression of SUMO pathway components? Although the nucleus is the primary location of sumoylation, it is clear that sumoylation may take place anywhere in the cell. SUMOs seem to serve as legal organizers and managers of distinct communities of the substrate proteins within the nucleus or cytoplasm of the cell. Do free SUMO proteins and their modifying enzymes shuttle in the cell and how is their shuttling regulated? Many sumoylated proteins localize to intracellular locations distinct from those of their non-sumoylated counterparts. Does the sumoylation occur first or does the substrate relocalize first? With the very limited number of SUMO E3 ligases, in contrast to that of ubiquitin E3 s, how is the substrate specificity of sumoylation precisely achieved? Do these few E3 s have to shuttle constantly between different sumoylation foci, or are there many more unknown SUMO E3 s to be discovered? How is SUMO signaling deregulated in pathologies? Studies in the years to come will certainly generate exciting answers to many of these questions.

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