Concepts in sumoylation: a decade on

Ruth Geiss-Friedlander and Frauke Melchior

Abstract | A decade has passed since SUMO (small ubiquitin-related modifier) was discovered to be a reversible post-translational protein modifier. During this time many enzymes that participate in regulated SUMO-conjugation and -deconjugation pathways have been identified and characterized. In parallel, the search for SUMO substrates has produced a long list of targets, which appear to be involved in most cellular functions. Sumoylation is a highly dynamic process and its outcomes are extremely diverse, ranging from changes in localization to altered activity and, in some cases, stability of the modified protein. At first glance, these effects have nothing in common; however, it seems that they all result from changes in the molecular interactions of the sumoylated proteins.

SUMO-interaction/binding motif
A short motif in proteins that mediates non-covalent interaction with SUMO. This motif is characterized as hxhh or hhxh (in which h is Val, Ile or Leu and x is any amino acid), flanked by acidic amino acids, and in some cases by Ser residues.

SUMO (small ubiquitin-related modifier) was identified as a reversible post-translational protein modifier a decade ago. Its discovery occured as a three-step process. The SUMO gene (*SMT3*) was initially identified in *Saccharomyces cerevisiae* in a genetic screen for suppressors of the centromeric protein Mif2 (REF. 1). Initial characterization of the protein came from three studies that discovered SUMO as a binding partner for human RAD51 and RAD52 (REF. 2), FAS (also known as Apo)[3] and PML[4] in yeast two-hybrid assays. Finally, SUMO was found covalently attached to the Ran GTPase-activating protein RanGAP1 (REFS 5,6). These studies showing covalent attachment demonstrated two important points: SUMO is a reversible protein modifier, and sumoylation can alter the localization of the modified target by altering protein interactions. Whereas unmodified RanGAP1 is cytosolic, sumoylated RanGAP1 localizes to the nuclear pore via interaction with the nucleoporin RanBP2 (also known as NUP358).

The discovery of sumoylation led to a burst of papers that reported the characterization of the enzymes involved in reversible modification (see below) and described, in rapid succession, many different target proteins. Hundreds of SUMO targets are now known, the majority of which are nuclear proteins. A consensus acceptor site was rapidly defined (see below), the mutation of which is now used as a tool to understand the outcome of target modification. The consequences of sumoylation for a target are impossible to predict, as modification can alter localization, activity or stability. A closer look suggests that the underlying principle of sumoylation is the alteration of inter- or intramolecular interactions of the modified substrate. Downstream consequences are mediated, at least in part, by effectors with non-covalent SUMO-binding motifs, the first of which has now been defined as SUMO-interaction/binding motif (SIM/SBM).

Here we summarize the current knowledge and emerging concepts of sumoylation. A general description of SUMO proteins is followed by details of the enzymes involved (for a list of proteins involved in sumoylation, see Supplementary information S1,S2 (tables)). We then describe the molecular outcomes of substrate sumoylation along selected examples and discuss an observation that is characteristic of all dynamic modifications: although modification appears to involve only a small proportion of a target protein, the effects can be dramatic. This review focuses on elucidating common mechanisms and principles that govern sumoylation, rather than specific functions. The different roles that SUMO has in transcription, nucleocytoplasmic trafficking, viral function and other processes have been covered elsewhere[7–13].

The SUMO family

SUMO proteins are ~10 kD in size and resemble the three-dimensional structure of ubiquitin[14–16]. However, they share less than 20% amino-acid sequence identity with ubiquitin and are different in their overall surface-charge distribution. All SUMO proteins carry an unstructured stretch of 10–25 amino acids at their N termini that is not found in any other ubiquitin-related proteins. The formation of SUMO chains is the only function that has been assigned to these N-terminal extensions (see below).

SUMO proteins are ubiquitously expressed throughout the eukaryotic kingdom. Some organisms, such as yeast, *Caenorhabditis elegans* and *Drosophila melanogaster*, have a single SUMO gene (Supplementary information S1 (table)). Other organisms, such as plants and vertebrates, have several SUMO genes. The human genome encodes four distinct SUMO proteins: SUMO1–SUMO4 (REFS 17,18).

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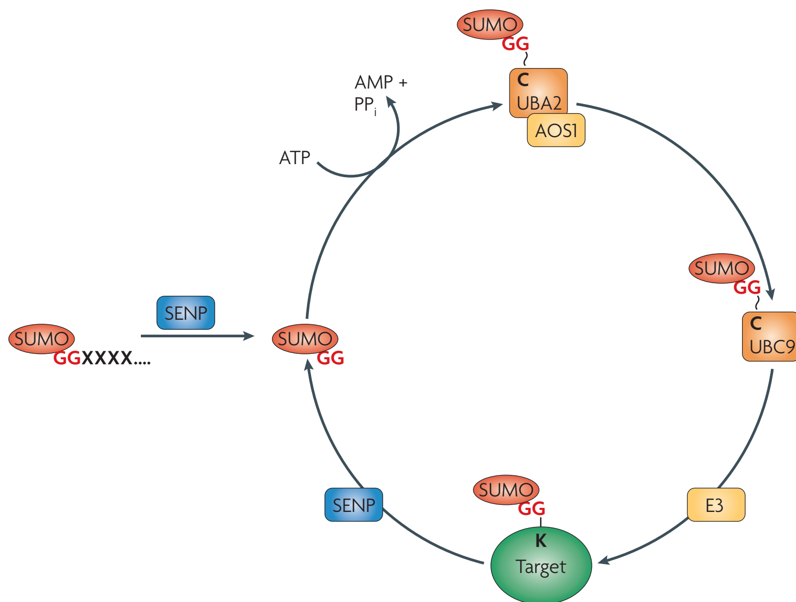

Figure 1 | **The mechanism of reversible sumoylation.** Before the first conjugation, nascent SUMO (small ubiquitin-related modifier) needs to be proteolytically processed to reveal its C-terminal Gly-Gly motif. This is accomplished by SUMO-specific isopeptidases (sentrin-specific proteases; SENPs), which remove 4 C-terminal amino acids from SUMO1, 11 amino acids from SUMO2 and 2 amino acids from SUMO3. Whether nascent SUMO4 can be processed to the mature form *in vivo* is currently unknown (see main text). Mature SUMO is activated by the E1 heterodimer AOS1–UBA2 in an ATP-dependent reaction, which results in a thioester bond between the C-terminal Gly residue and C173 in UBA2. SUMO is then transferred to the catalytic Cys residue of the E2 enzyme UBC9. Finally, an isopeptide bond is formed between the C-terminal Gly residue of SUMO and a Lys residue in the substrate. This step is usually aided by an E3 ligase. Sumoylated targets serve as substrates for SENPs, which ensures the reversible and dynamic nature of sumoylation.

E1 activating enzyme
An enzyme that forms a high-energy bond (thioester) with the C-terminal Gly residue of ubiquitin or a ubiquitin-like protein in an ATP-dependent reaction.

E2 conjugating enzyme
An enzyme that accepts ubiquitin or a ubiquitin-like protein from an E1 enzyme and transfers it to a substrate protein via the formation of an isopeptide bond. This step usually requires cooperation with an E3 ligase.

E3 ligase
An enzyme that facilitates the transfer of ubiquitin or ubiquitin-like protein from an E2 enzyme to a substrate protein. Ubiquitin HECT E3 ligases form thioester intermediates with ubiquitin, whereas all other known E3 ligases form complexes with the thioester-charged E2 and the target.

Of these, SUMO1–SUMO3 are ubiquitously expressed, whereas SUMO4 seems to be expressed mainly in the kidney, lymph node and spleen[17].

All SUMO proteins are expressed in an immature pro-form, in which they carry a C-terminal stretch of variable length (2–11 amino acids) after an invariant Gly-Gly motif that marks the C terminus of the mature protein. Removal of this C-terminal extension by SUMO-specific proteases is a prerequisite for the conjugation of SUMO to targets. The expression of the peptidic modifiers as precursors appears to be a common characteristic of ubiquitin-like modifiers. Whether maturation is a regulated or constitutive process remains to be shown. The mature forms of SUMO2 and SUMO3 are 97% identical, but share only 50% sequence identity with SUMO1. SUMO1 and SUMO2/3 serve distinct functions, as they are conjugated to different target proteins *in vivo*[19–21]. The role of SUMO4 remains enigmatic, as it is presently unclear whether it can be processed to its mature conjugation-competent form *in vivo*[17,22].

Sumoylation is an essential process in most organisms, including *S. cerevisiae*[23], *C. elegans*[24], *Arabidopsis thaliana*[25] and mice[26]. An exception seems to be fission yeast, in which disruption of the single SUMO gene *pmt3* leads to sick, but nevertheless viable, cells[27]. Whether individual SUMO proteins are essential in organisms that have multiple SUMO proteins remains to be seen. However, disruption of SUMO1 in mice causes embryonic lethality, and SUMO1 haploinsufficiency induces a developmental defect (split lip and palate) in mice and possibly in humans[28].

The mechanism of reversible sumoylation

Like ubiquitylation, sumoylation results in the formation of an isopeptide bond between the C-terminal Gly residue of the modifier protein and the ε-amino group of a Lys residue in the acceptor protein. Both ubiquitylation and sumoylation require an enzymatic cascade that involves three classes of enzymes. However, there is no overlap in the enzymes that are used in the conjugation cascade.

Attaching SUMO to substrates. The reversible sumoylation pathway is outlined in FIG. 1. The first step is the activation of a mature SUMO protein at its C terminus by the SUMO-specific E1 activating enzyme heterodimer AOS1–UBA2 (REFS 23,29–31). This reaction uses ATP for the formation of a SUMO–adenylate conjugate, which functions as an intermediate in the formation of a thioester bond between the C-terminal carboxy group of SUMO and the catalytic Cys residue of UBA2. Next, SUMO is transferred from UBA2 to the E2 conjugating enzyme UBC9, forming a thioester linkage between the catalytic Cys residue of UBC9 and the C-terminal carboxy group of SUMO[32–35]. Finally, UBC9 transfers SUMO to the substrate: an isopeptide bond is formed between the C-terminal Gly residue of SUMO and a Lys side chain of the target. This process is usually facilitated by SUMO E3 ligases, which are enzymes that catalyse the transfer of SUMO from UBC9 to a substrate.

The largest group of SUMO E3 ligases is characterized by the presence of an SP-RING motif, which is essential for their function[36]. This domain is predicted to resemble the RING domain in ubiquitin RING E3 ligases. SP-RING ligases bind their targets and UBC9 directly, and bind SUMO non-covalently via a SIM/SBM. Thus, they might function as platforms that position the target and thioester-charged UBC9 in a favourable orientation for SUMO transfer. SP-RING ligases can be subdivided into distinct groups, the first of which consists of PIAS family proteins, known as Siz proteins in yeast. Members of this family share a conserved ~400-amino-acid N-terminal domain in addition to the SP-RING[37]. Two PIAS family members have been described in *S. cerevisiae* (Siz1 and Siz2 (REFS 38,39)) and five have been described in mammals (PIAS1, PIAS3 and the splice variants PIASxα, PIASxβ and PIASy[40–46]). Other SP-RING ligases include MMS21 (also known as NSE2), which is part of an octameric SMC5–SMC6 complex that is essential for vegetative growth and DNA repair[47–49], as well as for telomerase-independent mechanisms of telomere lengthening in ALT cells[50]. An additional SP-RING E3 ligase is the meiosis-specific yeast protein Zip3, which is part of the synapsis-initiation complex[51].

The vertebrate-specific nuclear pore protein RanBP2 represents a second type of SUMO E3 ligases[52]. In contrast to SP-RING E3 ligases, RanBP2 has no known counterpart in the ubiquitylation cascade. The minimal catalytic domain of RanBP2 is natively unfolded and

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SP-RING motif
A RING-related sequence ($Sx_2Cx_{15}CxHx_2C/Sx_{17}Cx_2C$ (in which x is any amino acid)) that is predicted to have a RING-like structure.

RING domain
A sequence of Cys and His residues that binds two zinc cations: $Cx_2Cx_{(9-39)}Cx_{(1-3)}Hx_{(2-3)}C/Hx_2C/x_{(4-48)}Cx_2C$ (in which x is any amino acid).

PIAS family
A group of SUMO E3 ligases, initially identified for their ability to repress the transcription factor STAT3 (PIAS: protein inhibitors of activated STAT). All PIAS proteins share a SAP domain (which binds nucleic acids), an SP-RING and a SUMO-interaction/binding motif.

Polycomb group (PcG) proteins
A family of proteins, originally described in *Drosophila melanogaster,* that maintains the stable and heritable repression of several genes, including the homeotic genes.

Sentrin-specific proteases
(SENPs). Mammalian Cys proteases related to *Saccharomyces cerevisiae* Ulp1 and Ulp2. Like their yeast counterparts, most SENPs are SUMO-specific isopeptidases and C-terminal hydrolases (SENP8 is an exception).

Nuclear pore complex
(NPC). A macromolecular protein complex that is embedded in the nuclear envelope. NPCs allow the exchange of ions, metabolites and macromolecules between the nucleus and the cytoplasm.

SUMO-acceptor site
The Lys residue in a target to which SUMO is coupled. It is frequently found in the sequence motif ΨKxE (in which Ψ is a bulky aliphatic amino acid and x is any amino acid).

assumes its structure only upon folding around UBC9 (REFS 53,54). RanBP2 also interacts with SUMO, and it seems to accelerate catalysis by positioning the SUMO–UBC9 thioester for optimal attack by an acceptor Lys residue[53–55]. *In vivo* targets for RanBP2 are not yet known. However, *in vitro* it specifically enhances sumoylation of proteins such as histone deacetylase HDAC4, Sp100 and PML, but not of its binding partner RanGAP1 (REFS 52,55,56).

The human Polycomb group member Pc2 has been described as a third class of SUMO E3 ligases. Polycomb group (PcG) proteins form large multimeric complexes (PcG bodies) that are involved in gene silencing. Pc2 recruits the transcriptional co-repressor CtBP to PcG bodies. Overexpression of Pc2 results in enhanced CtBP sumoylation, and recombinant Pc2 enhances CtBP1 and CtBP2 modification *in vitro*[57,58]. Whether Pc2 functions as a true catalyst in multiple rounds of SUMO transfer from the E2 to its target, or whether it stimulates modification, for example, as a stoichiometric binding partner of CtBP, remains an open question. A recent addition to the list of factors that stimulate sumoylation is HDAC4. This protein, which is itself a SUMO target[56], has been shown to enhance sumoylation of myocyte-specific enhancer factor-2 (MEF2), LXRβ and the tumour suppressor HIC1 when overexpressed, and to enhance sumoylation of MEF2 that has been translated *in vitro* in a test tube[59–62]. As HDAC4 binds UBC9 and does not always require its deacetylase activity for enhanced sumoylation[59], other effects including E3 ligase activity have to be considered.

In most cases, sumoylation results in the addition of single SUMO entities to individual acceptor Lys residues. However, the formation of polySUMO chains (as is regularly found with ubiquitin) has been observed both *in vivo* and *in vitro*. This is best documented for *S. cerevisiae* SUMO (Smt3) and for mammalian SUMO2/3 (REFS 51,63–65). Although the function of SUMO chains is largely unknown, yeast cells that express SUMO mutants that cannot form chains are deficient in formation of synaptonemal complexes and in completion of meiosis (sporulation)[51].

Removing SUMO from substrate. Owing to the action of specific proteases, sumoylation is a reversible modification. So far, a single gene family that encodes SUMO-specific Cys proteases has been identified. The corresponding proteins are Ulp1 and Ulp2 in yeast[66,67], and the six Ulp homologues in humans are called sentrin-specific proteases (SENP1–3 and SENP5 –7). In addition to their isopeptidase function, Ulp/SENP proteins possess C-terminal hydrolase activity that is needed for the maturation of newly synthesized SUMO proteins (see above and FIG. 1). Members of the SENP family differ in their activity in maturation and isopeptide cleavage and also in their activity towards different SUMO paralogues: for example, SENP3 and SENP5 preferentially deconjugate SUMO2/3 from substrates[68,69]. Finally, SENPs vary in their predominant *in vivo* localization: *S. cerevisiae* Ulp1 and mammalian SENP2 are enriched at nuclear pore complexes[70–72]; SENP5 is enriched in the nucleolus[68,73], although a small fraction is also found in the cytoplasm and is required for mitochondrial fission and fusion[74]; SENP1 appears to shuttle between the cytoplasm and the nucleus[75,76]; and SENP6 has been reported to be both in the nucleus[65] and the cytoplasm[77]. For a recent overview on isopeptidases, see REF. 78.

SUMO-acceptor sites

The identification of a consensus SUMO-acceptor site was possible after mapping acceptor Lys residues in just a few SUMO targets, which included RanGAP1 (REFS 79,80), PML[81], Sp100 (REF. 82), inhibitor of nuclear factor-κB (IκBα)[83], p53 and c-Jun[84]. The SUMO-acceptor site was shown to be ΨKxE (in which Ψ is an aliphatic branched amino acid and x is any amino acid). This is remarkable because ubiquitylation-acceptor sites have not yet been defined. The reason for this is due, at least in part, to there being a single E2 enzyme (UBC9) that is responsible for transferring SUMO to all acceptor proteins; by contrast, more than 20 different E2s and many HECT-domain E3 ligases are responsible for ubiquitin transfer. A crystal structure of the complex between UBC9 and the C-terminal domain of RanGAP1 reveals how UBC9 interacts with this consensus motif: the target Lys residue reaches into the catalytic pocket of UBC9, whereas the aliphatic and acidic amino acids interact with residues on the surface of UBC9 (REF. 16). Recognition of the consensus-site motif by UBC9 is only possible if it is part of an extended loop, as in RanGAP1 (REF. 16), or present in an unstructured area, as in the transcription factor ETS1 (REF. 85) or the N termini of SUMO2/3 (REF. 63). UBC9 does not recognize consensus sites in stable helical structures[86].

Two different extensions of the simple consensus SUMO-acceptor site have recently been identified. The first is the phosphorylation-dependent sumoylation motif (PDSM), which is found in heat shock factor-1 (HSF1), Smad nuclear interacting protein-1 (SNIP1), MEF2 and several other proteins[87]. This motif consists of the conventional sumoylation motif followed by a phosphorylated Ser and a Pro residue (ΨKxExxpSP). Because the mutation of Ser to Asp enhances sumoylation of HSF1 *in vitro*[88], the additional negative charge of the phosphate group might enhance substrate–UBC9 interaction. This finding, which may well be expanded to include other phosphorylation sites, provides an attractive mechanism for regulated sumoylation (see below). An important role for negatively charged residues C-terminal of the acceptor Lys residue has been revealed by sequence analysis of many SUMO targets. This led to the definition of a second extended motif, the negatively charged amino-acid-dependent sumoylation motif (NDSM)[89]. A common theme for both motifs is that negative charge next to the basic SUMO consensus site enhances sumoylation.

Sumoylation sites of the ΨKxE type are found in many targets. However, some acceptor sites have been identified that do not contain this motif. Among these are residue K164 in *S. cerevisiae* proliferating cell nuclear antigen (PCNA), which is part of a hairpin turn sequence, and residue K14 in human E2-25K, which is part of a stable α-helix[86,90]. Whether these types of sites are rare exceptions or frequent occurrences and how UBC9 recognizes these sites are interesting questions for future studies.

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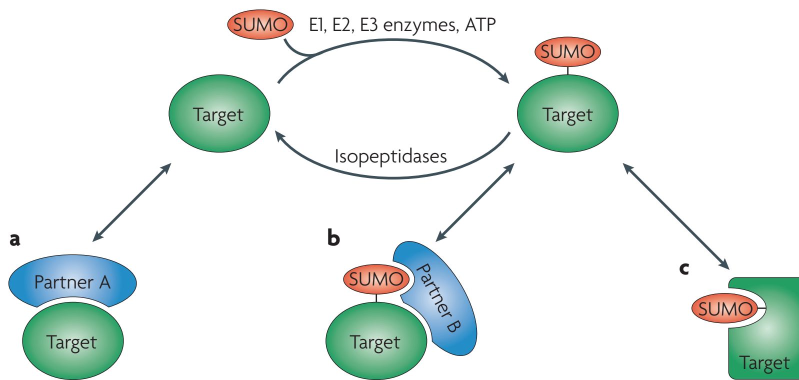

Figure 2 | **Molecular consequences of sumoylation.** Sumoylation can have three general consequences for a modified protein. **a** | Sumoylation can interfere with the interaction between the target and its partner, in which case the interaction can only occur in the absence of sumoylation. **b** | Sumoylation can provide a binding site for an interacting partner, for example via a so-called non-covalent SUMO (small ubiquitin-related modifier)-interaction/ binding motif (SIM/SBM). SIM/SBM is composed of a hydrophobic core that is flanked by acidic amino acids and, in some cases, by Ser residues. **c** | Sumoylation can result in a conformational change of the modified target. If the modified target also contains a SIM/SBM, intramolecular interaction between SUMO and the SIM/SBM can lead to a conformational change. So far, this has only been reported for one target, thymine DNA glycosylase (see main text and FIG. 4).

Molecular consequences of sumoylation

There is no simple way to predict what the functional consequence of the sumoylation of a given target will be. *In vivo*, sumoylation may influence any single aspect of a target protein, including stability, localization or activity. At the molecular level, sumoylation alters protein surfaces and thereby influences interactions with other macromolecules (FIG. 2). Consequently, sumoylation can promote protein–protein interactions: sumoylated RanGAP1 can interact with RanBP2 (REFS 5,6); sumoylated p300 interacts with HDAC6 (REF. 91); and sumoylated PCNA recruits the yeast DNA helicase Srs2 to replication forks[92,93]. On the other hand, sumoylation can also interfere with protein interactions: the SUMO-acceptor site of transcription repressor ZNF76 overlaps with its binding site for the TATA-binding protein[94]; upon sumoylation, CtBP loses its interaction with the PDZ domain of nNos[95]; and sumoylated E2-25K cannot interact with the ubiquitin E1 enzyme[86].

Conceptually, changes in protein–protein interactions after sumoylation may be due to simple masking of existing binding sites, addition of interfaces that are present in SUMO itself (see below), or conformational changes in the target that reveal or destroy existing binding sites. Insight into the molecular consequences of sumoylation is provided by a small number of nuclear magnetic resonance (NMR) and X-ray crystal structures of unmodified and sumoylated target proteins. These studies reveal that sumoylation of RanGAP1, E2-25K and ETS1 does not influence the structure of either the sumoylated target or of the SUMO modifier itself[54,85,86,96]. Consequently, for these targets the loss or gain of interactions seems to be due to simple masking or addition of binding sites. However, a more complicated situation appears to exist for thymine DNA glycosylase (TDG), as revealed by detailed biochemical analysis[97] and by the X-ray structure of a sumoylated TDG fragment[98]. Modification by SUMO1 induces a conformational change in TDG that results in a loss of DNA binding. The conformational change is mediated by an interaction of covalently attached SUMO with a non-covalent SUMO-binding site in TDG[98] (see below). In conclusion, the molecular consequences of sumoylation are target specific, and can positively or negatively influence interactions with proteins, DNA and other macromolecules.

Non-covalent interactions with SUMO

Sumoylation frequently allows novel interactions of the modified target. In the absence of conformational changes, novel interactions are only possible if SUMO itself contributes to the association with a downstream effector (FIG. 2c). Recently, a short motif was identified in proteins that interact non-covalently with SUMO. This motif, called SIM/SBM, is hydrophobic in nature. The hydrophobic core is flanked N- or C-terminally by acidic and/or Ser residues[99–102]. A possible role for Ser residues in the SIM/SBM is to regulate SUMO binding by phosphorylation, as negative charge strengthens SUMO binding[99–102].

NMR and X-ray studies revealed that the SIM/SBM motif forms a β-strand that binds in a parallel or anti-parallel orientation between the α-helix and a β-strand of SUMO[54,100,101,103]. What ensures specificity in the association of a SIM/SBM-containing protein with a particular sumoylated target? The affinity between SUMO and a SIM/SBM is in the high micromolar range[101,103], which is not surprising as the interface is small. High-affinity association could result from sumoylation, for example, if the target contains a second low-affinity interaction site for the SIM/SBM-containing protein (FIG. 2).

The study of SIM/SBM-containing proteins is young, and few proteins have as yet been shown to possess this motif. Among these are p73α[99], PML[99,100,104] and the transcriptional repressor Daxx[105], all of which are themselves sumoylated. Studies on Daxx suggest that the SIM/SBM has multiple functions: not only does Daxx lose its transrepression capability upon mutation of the SIM/SBM, which supports the previous finding that Daxx requires non-covalent SUMO interaction for repression[106], the mutation also abolishes Daxx sumoylation[105]. The SIM/SBM of PML is necessary for the nucleation and formation of PML bodies[104]. SIM/SBMs are also found in SUMO enzymes such as UBA2, PIAS E3 ligases and RanBP2 (REFS 54,100), in which they seem to contribute to enzyme function.

An exciting role for SIM/SBMs in recruiting a downstream effector was recently shown for a family of ubiquitin E3 ligases: RNF4 in mammals and the heterodimers Hex3–Slx8 and Rfp1–Slx8 in *S. cerevisiae* and *S. pombe*, respectively. These ligases specifically recognize artificial SUMO-containing substrates via their SIM/SBMs. Together with other data, this opens up the intriguing possibility that sumoylation serves to target selected proteins for degradation[107–110]. Considering the large number of SUMO targets, it seems likely that additional SUMO-interacting motifs and/or domains will be identified.

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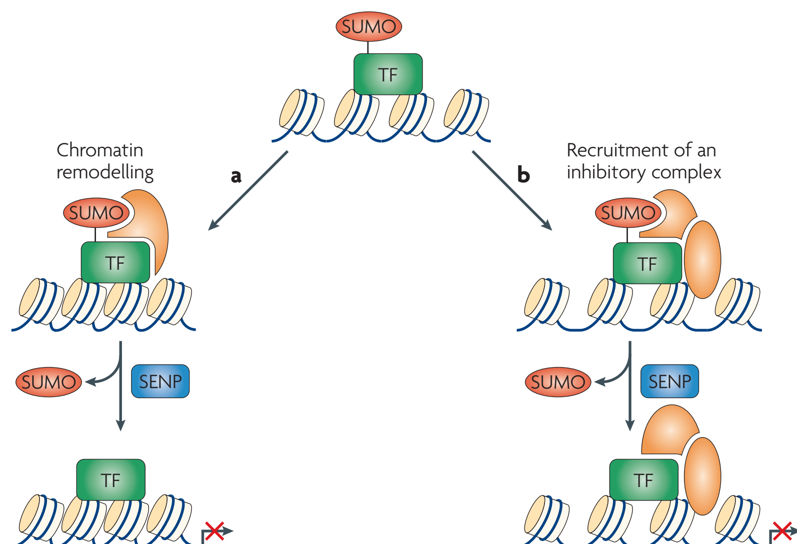

Figure 3 | **Low-level transcription factor sumoylation can result in quantitative repression. a** | Sumoylation of a transcription factor (TF) can allow recruitment of downstream repressive effectors that act enzymatically on the chromatin state (for example, chromatin-remodelling factors). Repressive changes in the chromatin structure (such as deacetylation) remain after desumoylation of the transcription factor by SUMO (small ubiquitin-related modifier)-specific isopeptidases (sentric-specific proteases; SENPs). **b** | Sumoylation of a transcription factor can initiate the recruitment of inhibitory factors to the promoter. After a stable repressor complex has formed, SUMO can be removed by SENP without affecting the complex.

Support for this idea is found in the ubiquitin system, in which many different ubiquitin-binding domains are known (reviewed in REF. 111).

Low-level sumoylation, big effect?

Only a few proteins are quantitatively sumoylated, either constitutively or upon receiving their respective upstream signals. Instead, most targets appear to be modified to a small percentage at steady state. How then does a small pool of sumoylated protein cause the dramatic effects that have been assigned to sumoylation? It is important to keep in mind that targets can undergo rapid cycles of modification and demodification. Although the equilibrium might lie on the side of the unmodified form, the whole pool of a given protein might be affected by sumoylation in a short window of time. Here we discuss two examples of how low-level sumoylation can cause large effects, both in gene transcription and in base-excision DNA repair.

Sumoylation and inhibition of transcription. Sumoylation can have a negative role in transcription (reviewed in REFS 7,12). Despite the fact that steady-state sumoylation is usually less than 5%, most transcription factors and transcriptional co-regulators become significantly activated when the SUMO acceptor Lys residue has been mutated to an Arg residue. As revealed by an extensive mutagenesis study, repression seems to be due to SUMO-dependent recruitment of downstream effectors — it requires an area on SUMO that is essential for interaction with SIM/SBMs[112] (see above). Candidate factors that have been implicated in SUMO-dependent gene repression are HDACs[91,113] and Daxx, for which repressive function in transcription is dependent on its interaction with sumoylated partners[105,106,114].

At least two repressive mechanisms are consistent with low steady-state levels of transcription-factor sumoylation. First, sumoylation of the transcription factor can lead to the recruitment of repressive factors with chromatin-remodelling activity. If this occurs, a repressive chromatin state will remain even after sumoylation of the transcription factor is lost (FIG. 3a). Alternatively, sumoylation of a transcription factor can be necessary to initiate the formation of a repressive complex (FIG. 3b). According to this model (described in REF. 115), proteins that contain a SBM/SIM will be recruited to sumoylated transcription factors and then serve as platforms for the assembly of a repressor complex. This complex will remain stable even after SUMO is removed from the initial target. In both cases, the transcription factor or the regulator only needs to be sumoylated for a short period of time, during which gene silencing is initiated.

Sumoylation of the DNA-repair enzyme TDG. The role of SUMO for the DNA-repair enzyme TDG has been extensively studied. A combination of biochemical and structural work resulted in the following model (FIG. 4). First, TDG recognizes and binds to a mismatch on DNA and than excises the corrupted base. However, TDG gets stuck in the reaction cycle because it binds tightly to the reaction product, the abasic site[97]. Subsequent sumoylation solves the problem — it causes a conformational change in TDG that reduces DNA affinity and thereby allows release of the enzyme into the nucleoplasm[97,98]. Specific isopeptidases quickly remove SUMO, which enables TDG to bind the next DNA mismatch. Steady-state levels of sumoylated TDG indicate that there is only a short window of time between release of TDG into the nucleoplasm and recognition by a SUMO isopeptidase, relative to the overall time of the complete reaction cycle. Consequently, the steady-state level of sumoylated TDG will be low, although TDG sumoylation is required for every single enzymatic cycle.

A highly dynamic modification

Many proteins are modified by SUMO only following extra- and intracellular stimuli, such as cell-cycle position, nutritional state, heat shock or DNA damage. Here we briefly discuss regulatory mechanisms that can enhance or decrease the sumoylation of specific targets.

Regulation at the level of individual targets. In many cases, regulation of sumoylation seems to occur at the level of the target itself, and involves other post-translational modifications. For example, as described above, phosphorylation of the PDSM enhances sumoylation of proteins such as HSF1 and MEF2, probably owing to enhanced interaction with UBC9 (REF. 87). However, phosphorylation can also be a negative regulator for sumoylation, as has been reported for IκBα, p53, c-Fos and c-Jun[83,84,116,117]. In these cases, the underlying mechanisms have not yet been elucidated. One possible explanation could be that the SUMO-acceptor site becomes masked, another could be that the target becomes relocalized.

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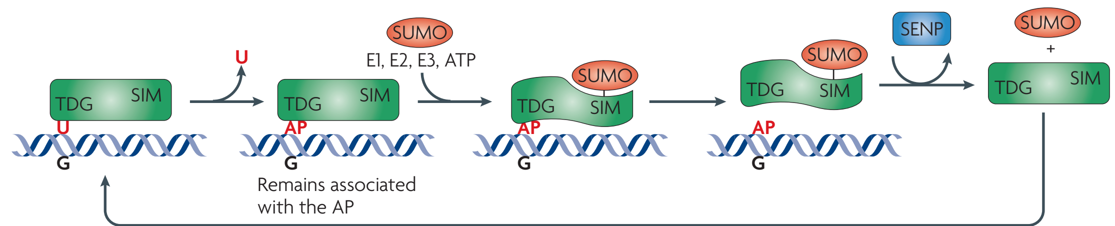

Figure 4 | **Thymine DNA glycosylase requires sumoylation and desumoylation for each catalytic cycle.** Thymine DNA glycosylase (TDG) functions in base-excision repair. It binds to G–U or G–T DNA mismatches and removes the mutated base to produce an abasic (AP) site. The enzyme has a high affinity for its product, the AP site, and requires a sumoylation-induced conformational change to be released. This conformational change is mediated via the interaction of SUMO (small ubiquitin-related modifier) with a SUMO-interaction motif (SIM; also known as a SUMO-binding motif (SMB)) in TDG. Once TDG is released, it is rapidly desumoylated by SUMO-specific isopeptidases (sentrin-specific proteases; SENPs) and can again bind with a high affinity to mismatches in the DNA. The model was first proposed in REF. 97 (see main text for further references).

In addition to phosphorylation, an obvious mechanism of regulation is competing modification of the acceptor Lys residue. Conceivable alternative modifications are acetylation, methylation and ubiquitylation. Several examples for the modification of specific Lys residues by either SUMO or ubiquitin (for example, in IκBα or PCNA[83,90]) or by SUMO and an acetyl group (for example, in Sp3, HIC1 or MEF2A[43,60,62,118]) have been described. Whether one modification serves to prevent the other (for example, if they occur on the same Lys residue) or whether both modifications are independently regulated events depends on the target in question.

Monoubiquitylation, polyubiquitylation and sumoylation of PCNA seem to be independently regulated events that depend on different upstream signals from DNA damage and cell-cycle position. DNA damage leads to mono- and polyubiquitylation of PCNA and, in turn, to error-prone or error-free DNA-damage repair. On the other hand, sumoylated PCNA associates with the helicase Srs2 during S phase to prevent DNA recombination[90,92,93,119] (reviewed in REF. 120). Consecutive modifications have been described for NEMO (nuclear factor (NF)-κB essential modulator): PIASy catalyses the modification of NEMO by SUMO1 (REF. 121), which, in turn, leads to phosphorylation by the nuclear kinase ATM. Subsequently, NEMO is desumoylated and ubiquitylated. This leads to translocation of NEMO to the cytoplasm, where it participates in the activation of NF-κB[122].

A truly competitive mechanism between sumoylation and acetylation was revealed in a recent study on the tumour suppressor HIC1, which can be sumoylated or acetylated on K314. Knockdown of the histone deacetylase SIRT1 results in increased acetylation and reduced sumoylation of HIC1 (REF. 62). A complex interplay between phosphorylation, acetylation and sumoylation occurs for MEF2A in the morphogenesis of postsynaptic granule neuron dendritic claws[123]. Activity-dependent calcium signalling induces the calcineurin-mediated dephosphorylation of MEF2A at residue S408, which promotes a switch from sumoylation to acetylation at K403.

Regulation of the enzymes. Target modification is only one mechanism of regulating sumoylation — changes in activity, abundance or localization of modifying enzymes and isopeptidases represent possible alternative mechanisms. The importance of regulated localization of an enzyme has been demonstrated for mitotic sumoylation of yeast septins. Septins are cytoskeletal proteins that are components of a belt of filaments around the bud neck. The responsible SUMO E3 ligase, Siz1, resides in the nucleus throughout interphase, but translocates to the bud neck during mitosis, where it sumoylates septins[38]. This sumoylation is not essential for the budding of yeast; however, yeast strains in which all seven sumoylation sites in three different septins have been mutated are deficient in the disassembly of the septin rings after cytokinesis[124].

The regulation of enzyme activity can affect individual target proteins, but it can also exert global effects on sumoylation. A dramatic increase in SUMO conjugation has been observed, for example, upon heat shock, osmotic stress and hibernation[19,125,126], and a loss of global SUMO conjugation has been observed under conditions of oxidative stress[127] or upon transfection of the protein Gam1 from the avian CELO adenovirus[128,129]. Although the mechanisms for global increase in sumoylation are not yet known, a global decrease in sumoylation is caused by inactivation of SUMO-conjugating enzymes: H_2O_2 that is often generated during periods of oxidative stress reversibly oxidizes SUMO-conjugating enzymes[127], whereas viral Gam1 targets SUMO E1 for degradation by the proteasome. Gam1 interacts both with the E1 subunit AOS1 and with subunits of an E3 ubiquitin-ligase complex, which leads to ubiquitylation and degradation of the E1 enzyme[128,129]. These examples, together with reports that link altered enzyme expression to cancer, suggest that SUMO-enzyme regulation will be an exciting topic for future studies[130,131].

Sumoylation: not just a nuclear affair

The large body of published work that focuses on the role of SUMO in transcription, DNA repair, nuclear bodies and nucleocytoplasmic transport might give the impression that sumoylation is restricted to the nuclear compartment. This would have been a misconception even in the early days of the SUMO field, and recent developments point to many exciting roles of SUMO in the soluble phase of the cytoplasm, the plasma membrane, mitochondria and the endoplasmic reticulum

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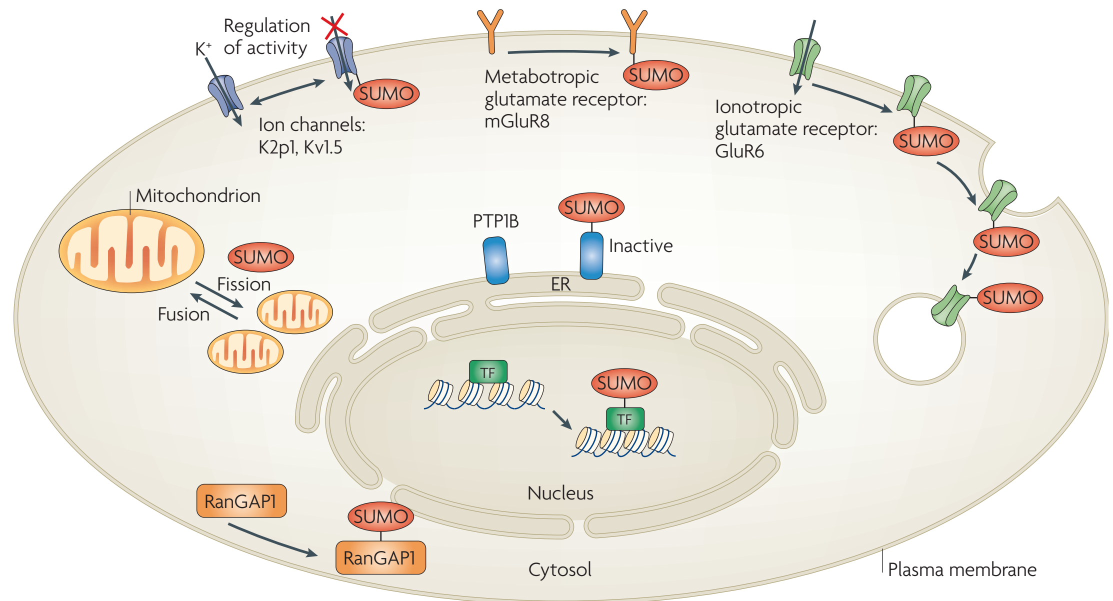

Figure 5 | **Sumoylated proteins are found throughout the cell.** Many known SUMO (small ubiquitin-related modifier) targets are nuclear proteins, such as transcription factors (TFs) and co-regulators or proteins that are involved in chromatin organization, replication and repair. However, SUMO targets are also found at the plasma membrane, the endoplasmic reticulum (ER) and in the cytoplasm. Selected examples are shown (see main text for references). RanGAP1 sumoylation leads to changes in its cellular localization: the unmodified protein is soluble in the cytosol, but is recruited to the nuclear pore complex after conjugation to SUMO1. The plasma-membrane-localized ion channels K2P1 and Kv1.5 appear to be less active upon modification. Sumoylation of the glutamate receptor GluR6 induces its internalization by as yet unidentified mechanisms. The effect of metabotropic glutamate receptor-8 (mGluR8) sumoylation is currently unknown. The ER-localized tyrosine phosphatase-1B (PTP1B) is inactivated by sumoylation. SUMO also affects mitochondrial dynamics: overexpression of SUMO or downregulation of the SUMO-specific isopeptidase SENP5 results in increased mitochondrial fission. Although the dynamin-like protein DRP1 is a target for sumoylation, it is currently unclear whether its modification is involved in this process.

(ER) (FIG. 5). A prerequisite for reversible sumoylation in a specific compartment is the availability of the enzymes required for modification and demodification. Although most of these enzymes are enriched in the nucleus, they are also present in the cytoplasmic compartment (reviewed in REF. 10). Consequently, it is presently not known whether proteins that shuttle between the nucleus and the cytoplasm, such as IκBα, are modified in the nucleus or the cytoplasm. This question is easier to answer for proteins with a restricted localization. Among these are some of the first-identified SUMO targets, the cytoplasmic protein RanGAP1 and yeast septins, which localize to the bud neck (see above).

Sumoylation and mitochondrial dynamics. Analysis of mitochondria fractions by western blot and immunofluorescence revealed the presence of sumoylated proteins in this compartment. A candidate is the known sumoylation target DRP1, a cytosolic dynamin-like GTPase that is involved in mitochondrial fission. Overexpression of SUMO1 or depletion of SENP5 by small interfering RNA results in fragmented mitochondria, which implies that reversible SUMO modification is necessary for maintaining the balance between mitochondrial fission and fusion[74,132]. The molecular details of how sumoylation affects mitochondrial dynamics remain to be elucidated.

Sumoylation at the ER membrane. The first-identified ER-associated SUMO target protein was protein-tyrosine phosphatase-1B (PTP1B). PTP1B is a ubiquitously expressed enzyme that localizes to the cytoplasmic face of the ER and the nuclear envelope through a targeting motif at its extreme C terminus. Interestingly, misplacement of PTP1B to the plasma membrane, the cytoplasm or the nucleoplasm reduces steady-state levels of sumoylation. Whether this is due to preferential modification or to reduced demodification at the ER remains to be seen. PTP1B negatively regulates growth-factor signalling and cell proliferation by dephosphorylating key receptor tyrosine kinases. Insulin treatment stimulates the sumoylation of PTP1B, which in turn impairs its activity, suggesting a positive role for SUMO in receptor tyrosine kinase signalling. How sumoylation inactivates PTP1B is currently unclear. Because sumoylation impairs the activity of the phosphatase *in vitro,* masking of the substrate-binding site or conformational changes that affect the catalytic pocket are likely explanations[133].

Sumoylation of plasma membrane proteins. Recently, SUMO was linked to channel and receptor regulation at the plasma membrane. Where mechanistic insights have been obtained, sumoylation appears to serve negative regulatory functions. However, molecular details are still scarce, and generalized hypotheses have to await further analysis.

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The first membrane protein that was reported to be sumoylated was the K2P1 potassium-leak channel. It seems to be inactive owing to quantitative sumoylation, which is remarkable considering that most proteins are sumoylated at only a small percentile at steady state (see above)[134]. However, a recent report casts doubts on whether the channel is indeed sumoylated[135], so this issue will have to be clarified in the future.

The plasma membrane voltage-gated potassium channel Kv1.5 was subsequently found to be regulated through reversible sumoylation[136]. As Kv channels play crucial roles in the highly regulated electrical responses throughout the cardiovascular system, these findings might have far-reaching medical implications. A candidate E3 ligase is PIAS3, as it was identified many years ago as a potassium-channel-associated protein that can modulate the surface expression and whole-cell current densities of several Kv isoforms[137]. Additional SUMO targets at the plasma membrane are the metabotropic glutamate receptor-8 (mGluR8)[138] and the GluR6 subunit of kainate receptor[139]. The *in vivo* function of mGluR8 sumoylation is currently unknown. By contrast, sumoylation of GluR6 is induced in response to kainite, and this modification appears to be a prerequisite for kainite-induced endocytosis of the receptor[139]. Although ubiquitin has a well-known role in vesicle trafficking, this finding provides an exciting first link between sumoylation and receptor-mediated endocytosis.

Conclusions and future directions

Sumoylation is an important and widely used reversible protein modification. Known targets are present at many cellular locations, ranging from the nucleus and cytoplasm to the ER and plasma membrane. Every conceivable consequence has been described for target sumoylation — it can inhibit transcription factors, affect the localization of proteins, inhibit or activate enzymes and target proteins for, or prevent, degradation. The underlying mechanism for these diverse effects is the alteration of inter- or intramolecular interactions of the modified target.

Not surprisingly, considering the young age of the field, many basic questions regarding components, mechanisms and consequences remain unanswered. For example, the list of characterized enzymes appears to be short considering the large number of target proteins that are modified in a regulated manner. Mechanisms such as alternative splicing and post-translational modifications are likely to contribute significantly to the presence of many functionally distinct SUMO E3 ligases and isopeptidases. Systematic analysis of splice-variant expression patterns, elucidation of individual target specificities and determination of the physiological roles (for example, by studying knockout animals) are important tasks for future studies. Identification of putative binding partners, which might contribute to target specificity and/or enzyme localization, will also significantly increase our understanding. Finally, there may be many as yet undiscovered SUMO enzymes around — which is not surprising considering that new ubiquitin proteases are still being discovered.

An incomplete picture also exists for the mechanism of paralogue-specific sumoylation. Although many targets show clear preference for SUMO1 or SUMO2/3 in cells, these targets are easily modified with all SUMO paralogues when using recombinant enzymes. This is true, for example, for RanGAP1, which is exclusively modified with SUMO1 *in vivo*. We seem to be missing the regulatory elements, such as co-factors or post-translational modifications, of these enzymes. Although the study on the formation of SUMO chains and their function is still young, recent developments suggest that this is yet another upcoming research area.

Our current knowledge on downstream effectors of mono- and polysumoylation is similarly limited. Currently, only one non-covalent SUMO-binding motif, known as SIM/SBM, has been characterized. As more than 20 different ubiquitin-binding motifs/domains have been identified for the ubiquitin pathway, the SUMO field may still have a lot to discover. Furthermore, what is the function of the global up- and downregulation of sumoylation that has been observed, for example, upon stress, hibernation or viral infection? Simultaneous loss or gain of many conjugates might serve to coordinate multiple pathways, for example, during a switch from proliferation to apoptosis, induction of differentiation or change in metabolism.

Although links to developmental defects and diseases are still limited and poorly understood (reviewed in REFS 130,140,141), it might only be a matter of time until altered sumoylation is identified as a cause for specific disorders. In conclusion, 10 years of SUMO research has led to the maturation of a truly exciting research field, but much remains to be discovered during the next decade.

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DATABASES
UniProtKB: http://ca.expasy.org/sprot
Daxx | MMS21 | RanBP2 | RanGAP1 | SENP1 | SENP2 | SENP3 | SENP5 | SENP6 | Siz1 | Srs2 | SUMO1 | SUMO2 | SUMO3 | SUMO4 | TDG | UBC9

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