SUMO, UBIQUITIN'S MYSTERIOUS COUSIN

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Covalent modification of cellular proteins by the ubiquitin-like modifier SUMO regulates various cellular processes, such as nuclear transport, signal transduction, stress response and cell-cycle progression. But, in contrast to ubiquitylation, sumoylation does not tag proteins for degradation, but seems to enhance their stability or modulate their subcellular compartmentalization.

UBIQUITIN AND PROTEASOMES

Post-translational modification of proteins is an important means to alter their function, activity or localization after their synthesis has been completed. In these cases, specific amino-acid residues of target proteins are chemically modified by various molecules, such as phosphate, acetate, lipids or sugars. Modification with ubiquitin represents a unique case because the modifier itself is a small polypeptide. Ubiquitin is usually attached to lysine side chains of target proteins, resulting in 'branched', ISOPEPTIDE-linked ubiquitin–protein conjugates (see review by Allan Weissman on page 169 of this issue). Conjugation of ubiquitin (termed ubiquitylation or ubiquitination) has a well-established role in earmarking proteins for degradation by the 26S PROTEASOME[1] (see review by Peter Kloetzel on page 179 in this issue). During the past few years, several proteins have been discovered that have sequence similarity to ubiquitin. These ubiquitin-like proteins fall into two separate classes. Proteins of the first class, termed 'ubiquitin-like modifiers' (UBLs) function as modifiers in a manner analogous to that of ubiquitin. Examples are SUMO (small ubiquitin-related modifier), Rub1 (also called Nedd8), Apg8 and Apg12 (see review by Yoshinori Ohsumi on page 211 of this issue). Proteins of the second class, including parkin, RAD23 and DSK2, are designated 'ubiquitin-domain proteins' (UDPs). These proteins bear domains that are related in sequence to ubiquitin but are otherwise unrelated to each other. In contrast to UBLs, these proteins are not conjugated to other proteins[2,3].

The UBL protein SUMO (also called sentrin) is present in all eukaryotic kingdoms[4] and is highly conserved from yeast to humans. Whereas invertebrates have only a single *SUMO* gene, which has also been termed *SMT3*, three members of the SUMO family have been described in vertebrates: SUMO-1 and the close homologues SUMO-2 and SUMO-3 (REF. 5). SUMO-1, the founding member of the family, is also known as PIC-1, sentrin or GMP1 (REFS 6–8). For simplicity, we use the term SUMO here for all members of the SUMO family, including those from yeast and flies. Human SUMO-1, a 101-amino-acid polypeptide, shares ~50% sequence identity with the closely related SUMO-2/SUMO-3 and with the yeast *Saccharomyces cerevisiae* Smt3 protein (FIG. 1). Although the overall sequence identity between SUMO-1 and ubiquitin is only about 18%, structure determination by nuclear magnetic resonance (NMR) revealed that both share a common three-dimensional structure that is characterized by a tightly packed globular fold with β-sheets wrapped around one α-helix[9]. SUMO has a short amino-terminal extension that is absent in ubiquitin. The function of this extension is unknown, but interestingly, the sequences of these extensions are different for the distinct SUMO members (FIG. 1). After the initial observation that SUMO-1 can be attached to the mammalian RanGAP1 protein[8,10] (see below), work in several laboratories has established that covalent modification of substrate proteins with SUMO represents a novel and widely used type of post-translational modification. But the functional significance of this modification remains a matter of debate.

ISOPEPTIDE BOND
Any amide bond formed between a carboxyl group of one amino acid and an amino group of another where either group occupies a position other than α.

26S PROTEASOME
Large multisubunit protease complex that selectively degrades multi-ubiquitylated proteins. It contains a 20S particle that carries the catalytic activity and two regulatory 19S particles.

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Figure 1 | **Sequence alignment of SUMO family members and ubiquitin.** The sequences of human SUMO-1, -2, and -3 are compared to SMT3 from *Saccharomyces cerevisiae* and to human ubiquitin. Identities are shown in blue, similarities in yellow. Precursor processing occurs carboxy-terminal to the double-glycine motif (scissors symbol).

CYSTEINE PROTEASE
Protease that has a cysteine at the active site.

Pathway of SUMO conjugation

The pathway of sumoylation is mechanistically analogous to ubiquitylation, but SUMO conjugation requires a set of enzymes that is distinct from that acting on ubiquitin (FIG. 2). Ubiquitin is synthesized as an inactive precursor protein, which has to be processed by a specific protease (called ubiquitin carboxy-terminal hydrolase) to make the carboxy-terminal double-glycine motif available for conjugation. Subsequently, ubiquitylation proceeds through a three-step mechanism. Ubiquitin is initially activated by the ubiquitin-activating enzyme E1 and then transferred to a reactive cysteine residue of one of several E2s (ubiquitin-conjugating enzyme or UBC). In most cases at least one additional factor, called E3 or ubiquitin–protein ligase, is required for the formation of an isopeptide bond between ubiquitin and the target protein. E3s are considered to be largely responsible for substrate specificity. Usually, several ubiquitin molecules are conjugated to a substrate in the form of a so-called 'multi-ubiquitin' chain and the resulting multi-ubiquitylated proteins are the preferred substrates of the 26S proteasome. Multi-ubiquitylation sometimes requires another factor termed E4 (REF. 11).

Like ubiquitin, all SUMO forms are initially made as inactive precursors. They mature by a carboxy-terminal proteolytic cleavage event, which yields the mature modifiers with exposed carboxy-terminal glycine residues. These residues are required for the formation of SUMO–protein conjugates, that is, the formation of an isopeptide bond between the carboxyl terminus of SUMO with an ε-amino group of a lysine residue of a target protein. The processing reaction is catalysed by a group of CYSTEINE PROTEASES, termed ULPs (ubiquitin-like protein-processing enzyme) or SUMO-specific proteases (see below). A SUMO-specific E1 activity has been purified and characterized in yeast and humans[12–14]. The protein is a heterodimer composed of the proteins AOS1 (SUA1) and UBA2 (SUA2). Remarkably, UBA2 bears clear sequence similarity to the carboxy-terminal region of UBA1, the E1 enzyme for ubiquitin, whereas AOS1 is related to the amino-terminal part of UBA1. The UBA2 subunit bears the 'active site' cysteine residue required for the formation of SUMO–E1 enzyme thioesters, but both subunits are required for SUMO activation *in vitro* and *in vivo*. SUMO has a single E2-type-conjugating enzyme, UBC9, which is specific for SUMO and does not act on ubiquitin[15–18]. A structural comparison of UBC9 with ubiquitin-specific E2 enzymes revealed, in spite of an overall similarity, important differences between these enzymes[19,20]. In particular, the surface of UBC9, which is involved in SUMO binding, is mainly positively charged, whereas the corresponding regions in ubiquitin-specific E2s (for example, UBC4 and UBC7) have negative or neutral potentials. These differences are likely to have a role in modifier discrimination. Notably, the positively charged binding surface of UBC9 is highly complementary in its electrostatic potentials and hydrophobicity to the negatively charged surface of SUMO. Ubiquitin cannot bind to UBC9 because it has positive charges in this region. UBC9 has been shown to physically interact with almost all known SUMO substrates in yeast two-hybrid assays, possibly indicating that UBC9 can be sufficient for substrate recognition. It seems more attractive to speculate, however, that, in analogy to the ubiquitylation process, substrate specificity of the SUMO-conjugation pathway might require additional, as-yet-unidentified E3-type ligases. In contrast to ubiquitin, SUMO conjugation does not seem to lead to the formation of SUMO–SUMO chains. This is consistent with the observation that the typical branched-point lysine residues (K29, K48, K63) of ubiquitin are not present in SUMO. Moreover, no mixed modifier chains that contain both SUMO and ubiquitin or other UBLs have been reported.

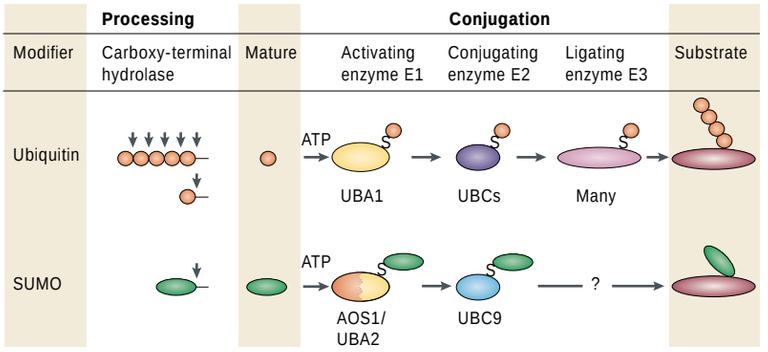

Figure 2 | **Conjugation pathway of ubiquitin and the ubiquitin-like modifier SUMO.** Ubiquitin and SUMO are synthesized as precursors and processed carboxy-terminally by hydrolases (vertical arrows) and are subsequently conjugated to proteins involving activating (E1) and conjugating (E2) enzymes that form thioesters (S) with the modifiers.

SUMO de-conjugation

Sumoylation is a dynamic, reversible process. The cleavage of SUMO from its target proteins, here termed 'desumoylation', is catalysed by ULP proteases. In yeast, two of these enzymes — Ulp1 and Ulp2 — have been identified[21–23]. *In vitro*, both Ulp1 and Ulp2 can catalyse the carboxy-terminal processing of SUMO and both enzymes can remove SUMO from isopeptide-linked conjugates. The sequence similarity of the two enzymes is restricted to a 200-amino-acid sequence called the ULP domain, which harbours the catalytically active region. The three-dimensional structure of the ULP domain from Ulp1 has been determined in a complex with the *S. cerevisiae* SUMO (Smt3) precursor[24] (FIG. 3). Interestingly, Ulp1 shares no sequence or structural similarity to de-ubiquitylating enzymes, although both belong to the cysteine protease superfamily.

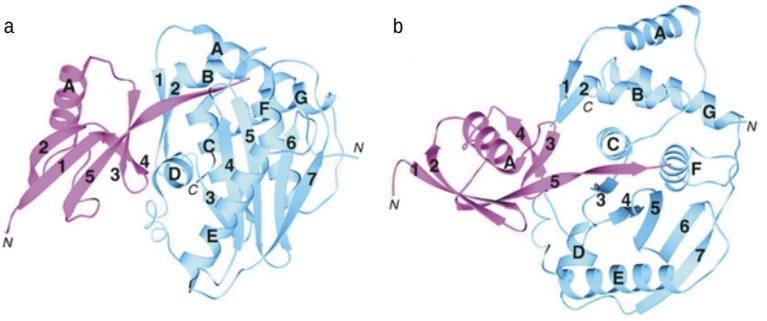

Figure 3 | **Structure of the ULP-1–SMT3 complex.** ULP-1 is shown in blue, SMT3 in purple. β-strands are numbered; α-helices are lettered. **a** | View looking onto one side of the complex. **b** | A perpendicular view of the complex looking onto the active site. SUMO does not undergo large conformational changes when bound to ULP1, but its carboxyl terminus adopts a more extended conformation in the complex that ends with a covalent bond between the terminal glycine residue and the catalytic cysteine residue (Cys580) of ULP1. The active site of ULP1 resides in a narrow cleft comprised by conserved amino acids that recognize the carboxy-terminal Gly-Gly-X motif of the SUMO precursor by van der Waals contacts. The structure of the active-site cleft of ULP1 allows the access of even large SUMO-protein conjugates. This is in striking contrast to de-ubiquitylating enzymes, where a bulky loop at a similar region allows only the access of small or denatured ubiquitin conjugates.

Like SUMO conjugation, SUMO de-conjugation is needed for viability in the budding yeast[21–23]. In this organism, only Ulp1, but not Ulp2, is essential for viability. SUMO conjugates accumulate in cells lacking either *ULP1* or *ULP2*. Interestingly, the SUMO CONJUGATE PATTERN is distinct for each of the individual mutants, indicating that the two enzymes act on distinct substrates. Consistent with that, *ULP2* overexpression cannot suppress the lethality of the *ULP1* deletion. Surprisingly, however, inactivation of *ULP2* partially rescues the defects caused by a *ULP1* deficiency, and the double mutant accumulates fewer SUMO conjugates than either of the single mutants. An expressed sequence tag (EST) databank search, based on the conserved ULP domain from yeast Ulp1, identified a family of mammalian ULP homologues. In humans at least 7 ULPs, with sizes ranging from 238 to 1,112 amino acids, were identified[25]. The enzymes were termed SENPs or SUSPs (for sentrin/SUMO-specific proteases). It is not yet known whether SUSPs have a dual role as maturation enzymes for SUMO and as isopeptidases for substrate de-sumoylation. With respect to the large number of mammalian SUSPs, it is reasonable to speculate that distinct enzymes might have specialized functions. Alternatively, the diversification in the mammalian SUMO de-conjugation system might be explained in part by the fact that higher eukaryotes express distinct SUMO forms (that is, SUMO-1, -2, -3). Indeed, a recently identified SUSP enzyme from mouse, Smt3-specific isopeptidase 1 (SMT3IP1), has a higher *in vitro* cleavage activity towards SUMO-2 conjugates than towards SUMO-1 conjugates[26]. What determines the substrate specificity of ULPs *in vivo* is not known, but studies in mammalian cells indicate that ULPs have different intracellular localization[26–28]. For example, the mammalian enzyme SENP-1 that localizes to the nucleus does not act on cytoplasmic SUMO–RanGAP1 conjugates but it removes SUMO from the nuclear substrate PML (promyelocytic leukaemia)[27]. However, other factors are likely to also be important, as the cytosolic SUSP-1 enzyme does not remove SUMO from RanGAP1 (REF. 28). Further studies of ULP-family members are expected to show that SUMO de-conjugation has important regulatory roles, especially in more complex organisms.

SUMO CONJUGATE PATTERN
Pattern of bands corresponding to sumoylated substrates detectable on an immunoblot with an anti-SUMO antibody.

EST
DNA sequence obtained by sequencing an end of a random complementary DNA clone from a cDNA library.

NUCLEAR PORE COMPLEX
Large multiprotein complex that forms a channel in the nuclear envelope of an eukaryotic cell, joining the inner and outer nuclear membranes and allowing transport of proteins to and from the nucleus.

BICOID
A segment polarity protein, discovered in *Drosophila*, that provides positional cues for the development of head and thoracic segments.

PML NUCLEAR BODIES
One type of nuclear speckles of unknown function that contains several proteins, including the promyelocytic leukaemia protein PML. PML nuclear bodies are also called PODs (PML oncogenic domains) or ND10 (nuclear dots 10).

Main functions of SUMO

SUMO has much fewer cellular substrates than ubiquitin but, intriguingly, several identified targets turned out to be important cellular regulators (TABLE 1; ONLINE FIG 1). But how the SUMO modification influences the function of the target proteins is not clear. In the following section, we focus on the main findings and describe two functional models for SUMO that are now being discussed in the field.

An address tag for protein targeting? The first substrate identified as a target for SUMO-1 was the Ran GTPase-activating protein RanGAP1, a component of the nuclear import machinery[8,10]. RanGAP1 is a key regulator of the Ras-like GTPase Ran, which controls nucleo-cytoplasmic transport[29]. Intriguingly, only the SUMO-1–RanGAP1 conjugate can stably interact with Ran-binding protein 2 (RanBP2), a protein located at the cytoplasmic face of the NUCLEAR PORE COMPLEX. This finding might indicate that sumoylation either targets RanGAP1 to the nuclear pore complex or, alternatively, stabilizes RanGAP1–RanBP2 complexes. The regions in RanGAP1 that determine sumoylation and RanBP2 binding, are non-overlapping. This indicates that SUMO does not directly mediate RanBP2 binding but rather induces a structural change in RanGAP1 that allows its binding to RanBP2 (REFS 30–32). Consistent with this, the free SUMO modifier does not directly interact with RanBP2.

The yeast homologue of RanGAP1, Rna1, lacks the carboxy-terminal tail region of mammalian RanGAP1 that harbours the sumoylation site and does not seem to be sumoylated[18]. However, the ULP1 protein has been shown to interact with components of the yeast nuclear pore complex[22,33,34], indicating that sumoylation in yeast might also have some function in certain nuclear transport pathways. Indirect evidence for a more general role of SUMO in nuclear import processes also comes from studies with a *Drosophila melanogaster* mutant that harbours a loss-of-function mutation in the *UBC9* gene (*semushi*)[35]. In these mutants, nuclear import of the transcription factor BICOID is prevented, leading to defects in embryogenesis. It remains to be determined whether this is directly mediated by impaired sumoylation of the *Drosophila* RanGAP1 protein.

Whereas RanGAP1 seems to be the main cytosolic substrate of SUMO, most sumoylated proteins are nuclear[36]. Most intriguingly, sumoylated substrates are often found in specific subnuclear protein complexes and preferential accumulation sites for sumoylated proteins are the so-called PML NUCLEAR BODIES (BOX 1). PML, the defining component of PML nuclear bodies, is a member of the family of RING-FINGER PROTEINS. It shows certain cell growth and tumour-suppressive properties and has

Table 1 | Known substrates for SUMO

Protein	Function	Role of sumoylation	References
Mammalian			
RanGAP1	Nuclear import	Mediates interaction with RanBP2	8,10,30–32
PML	Tumour supressor	Allows formation of NBs and recruitment of Daxx/p53 to NBs	39–50
Sp100	Chromatin remodelling (?)	Mediates interaction with HP1 (?)	40,58
p53	Tumour supressor	Activates p53 transactivation and apoptosis	52–54
p73	p53 homologue	Unknown	55
HIPK2	Transcriptional corepression	Mediates the localization of HIPK2 to nuclear dots	60
TEL	Transcriptional repression	Mediates the localization of TEL to nuclear dots	61,62
c-Jun	Transcriptional activation	Slightly reduces transcriptional activity of c-Jun	54
Androgen receptor	Transcriptional activation	Reduces transcriptional activity of androgen receptor	63
IκBα	Signal transduction, NF-κB inhibition	Inhibits ubiquitylation of IκBα Blocks NF-κB activity	65
Mdm2	E3 ubiquitin ligase for p53	Inhibits ubiquitylation of Mdm2 Acitvates the E3 function of Mdm2	66
Topo I	DNA replication, DNA repair	Unknown, induced after DNA damage with camptothecin	83
Topo II	DNA replication, DNA repair	Unknown, induced after DNA damage with teniposide	84
WRN	DNA helicase (RecQ family)	Unknown	85
RanBP2	Component of nuclear pore complex	Unknown	98
GLUT1	Glucose transport	Unknown, GLUT1 protein levels are downregulated by UBC9	99
GLUT4	Glucose transport	Unknown, GLUT4 protein levels are upregulated by UBC9	99
Drosophila			
Ttk 69	Transcriptional repression	Unknown	64
Dorsal	Signal transduction	Activates nuclear import of Dorsal	68
CaMK	Calcium/calmodulin-dependent kinase	Unknown	100
Yeast			
Septins	Bud-neck formation	Regulates dynamics of the neck ring	74,75
Viral			
CMV IE1	Immediate-early viral regulator	Unknown, correlates with the loss of PML sumoylation	45
CMV IE2	Immediate-early viral regulator	Decreases transactivation potential of IE2	93
EBV BZLF1	Immediate-early viral regulator PML sumoylation	Unknown, correlates with the loss of	94
HPV/BPV E1	DNA helicase Initiates viral replication	Regulates nuclear import of E1	101

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HPV/BPV, human papillomavirus/bovine papillomavirus; RanGAP1, Ran GTPase activating protein; IE, immediate-early; PML, promyelocytic leukaemia; RanBP2, Ran-binding protein 2; Topo, topoisomerase; Ttk, tramtrack.

a pro-apoptotic function. These activities are at least partially mediated by the potential of PML to act as a transcriptional co-activator in conjunction with the p53 tumour suppressor protein[37,38]. A fraction of PML undergoes sumoylation at three distinct lysine residues in the protein, and there is accumulating evidence that the sumoylation of PML regulates the assembly and/or stability of nuclear bodies[39–43]. Thus, the nuclear-body-containing detergent-insoluble cell fraction is highly enriched for SUMO–PML conjugates[39]. Disassembly of nuclear bodies by viral proteins (BOX 2) or during mitosis correlates with a complete loss of PML sumoylation[44–46]. Intriguingly, in PML-deficient mutant cell lines (*PML*$^{-/-}$) that cannot form nuclear bodies properly, reassembly of these structures can be induced by transfection with wild-type PML but not with a mutant version that lacks the sumoylation sites[47].

Although the function of PML nuclear bodies remains enigmatic, a number of examples show how effectively sumoylation of PML and subsequent recruitment of certain proteins to nuclear bodies can modulate transcriptional activity. Upon sumoylation of PML, the transcriptional corepressor Daxx relocalizes to nuclear bodies, where it seems to be stored in an inactive state[48–50] (FIG. 4). Sumoylation of PML also directs p53 to nulcear bodies but this leads to a stimulation of the transcriptional and pro-apoptotic activity of p53 rather than an inhibition[38]. It has been suggested that recruitment of p53 to nuclear bodies could trigger activating modifications in p53, such as acetylation[51]. Furthermore, evidence

RING-FINGER PROTEINS
A family of proteins structurally defined by the presence of the zinc-binding RING-finger motif. The RING consensus sequence is: CX2CX(9–39)CX(1–3)HX(2–3)C/HX2CX(4–48)CX2C. The cysteines and histidines represent metal binding sites. The first, second, fifth and sixth of these bind one zinc ion and the third, fourth, seventh and eighth bind the second.

Box 1 | PML nuclear bodies

PML (promyelocytic leukaemia) nuclear bodies (also known as ND10 or PODs) are distinct subnuclear structures of higher eukaryotes, which appear as dense spherical particles, 0.3–0.5 μm in diameter, that are tightly associated with the nuclear matrix[91,92]. Normal cells have between 10–30 PML nuclear bodies per nucleus. They all contain the nuclear body core components PML and Sp100 and a number of additional proteins that transiently associate with nuclear bodies. Among these are the transcriptional repressor Daxx, the tumour suppressors Rb and p53 and the Bloom syndrome protein (BLM). The assembly of nuclear bodies is highly dynamic and sensitive to environmental stimuli, such as response to stress or interferon. Furthermore, the integrity of PML nuclear bodies is compromised in certain pathological situations such as acute promyelocytic leukaemia and upon infection by certain DNA viruses. Although nuclear bodies have been implicated in a number of cellular processes, including the regulation of programmed cell death, transcription or antigen presentation, their main biological function remains largely enigmatic. Many sumolyated proteins are found in PML nuclear bodies, indicating that SUMO directs proteins to nuclear bodies or, alternatively, that nuclear bodies might support sumoylation.

is accumulating that nuclear bodies might stimulate SUMO conjugation, and that proteins that transiently associate with nuclear bodies include SUMO targets. Indeed, previous work has shown that p53 itself is a target for sumoylation[52–54]. The sumoylation sites in p53 are located within the carboxy-terminal region, which is known to regulate the DNA-binding activity of the protein. As a consequence, sumoylation of p53 moderately stimulates the transcriptional and apoptotic activity of the protein. It is attractive to speculate that the two findings are linked, that is, that the association of p53 with nuclear bodies is a prerequisite for p53 sumoylation. The significance of p53 sumoylation is underscored by the finding that the p53-related protein p73 is also covalently modified by SUMO at its extreme carboxy-terminal lysine residue[55]. Sumoylation of p73 does not notably alter its transcriptional properties but rather seems to be implicated in regulating its subcellular localization. Similarly to what is observed for PML, the SUMO–p73 conjugate is preferentially recovered in detergent-insoluble nuclear complexes. Whether these complexes represent PML nuclear bodies has not yet been determined.

A further link between the SUMO-modification system and nuclear bodies is suggested by the finding that the Sp100 protein, another core component of PML nuclear bodies, is also a major cellular substrate for sumoylation[40]. The precise function of Sp100 is unknown, but its interaction with chromosomal non-histone proteins of the HP1 and HMG1/2 families points to a role in chromatin organization[56,57]. A Sp100 mutant protein that lacks the SUMO attachment site can still localize to nuclear bodies, indicating that sumoylation of Sp100 is not required for targeting to nuclear bodies[58]. However, *in vitro*, sumoylated Sp100 has a higher affinity for the HP1 protein, indicating that sumoylation of Sp100 might directly mediate this interaction[59]. In light of the recent findings on PML, it will be interesting to see whether sumoylation of Sp100 recruits HP1 to the nuclear bodies and modulates its repressive function.

More recent data on the transcriptional regulators HIPK2 and TEL provide further evidence for a role of SUMO in regulating the subnuclear localization and transcriptional activity of proteins. HIPK2 is a serine/threonine kinase that physically interacts with HOMEODOMAIN TRANSCRIPTION FACTORS and acts as a transcriptional corepressor. Similarly to what is observed for PML, SUMO-1-conjugated HIPK2 forms are found in detergent-insoluble subnuclear complexes. The nature of these nuclear dots has not yet been determined, but the localization of HIPK2 to these dots depends on its sumoylation[60]. Similarly, TEL, an ETS-related transcriptional repressor, localizes to S-phase-specific nuclear dots in a SUMO-dependent manner, providing further evidence of a role for SUMO in regulating subnuclear localization[61]. Whether the modulation of nuclear localization by SUMO alters the repressive function of HIPK2 or TEL is unclear. However, overexpression of UBC9 relieves TEL-mediated repression, arguing for a modulation of TEL activity through sumoylation[62]. The regulation of transcription factors through sumoylation seems to be a recurring theme. Thus, in mammals the proto-oncogene c-Jun and the androgen receptor are sumoylated, and disruption of the SUMO-1 acceptor sites in these proteins enhances their transcriptional activity, indicating that sumoylation negatively regulates their transactivation potential[54,63]. In *Drosophila*, the transcriptional repressor Tramtrack 69 protein (Ttk69), which inhibits neuronal differentiation, was identified

HP1 FAMILY
(Heterochromatin protein 1 family). A family of chromosomal non-histone proteins primarily associated with heterochromatin. HP1 proteins have been implicated in gene regulation, DNA replication and nuclear architecture.

HMG1/2 FAMILY
(High-mobility group 1/2). Large protein family of small non-histone components of chromatin that function in higher-order chromatin structure.

HOMEODOMAIN TRANSCRIPTION FACTORS
Transcription factors with a 60-amino-acid DNA-binding domain comprised of three α-helices.

ETS
Proto-oncogene family related to v-ets, one of the oncogenes of the acutely transforming avian erythroblastosis virus E26.

Box 2 | Viral proteins — regulators of sumoylation?

The immediate-early proteins IE1 and IE2 from human cytomegalovirus and the BZLF1 (Z or Zebra) protein from Epstein–Barr virus are modified by SUMO[45,93,94]. Both IE1 and IE2 colocalize transiently in PML nuclear bodies at very early time points after infection. Subsequently, IE1 triggers the disaggregation of nuclear bodies, leading to a complete redistribution of nuclear body components into a diffuse nuclear pattern. Intriguingly, SUMO conjugation to IE1 is paralleled by the abrogation of PML sumoylation and thus correlates with the disassembly of nuclear bodies[45]. In striking analogy, sumoylation of BZLF1 is accompanied by the disappearance or desumoylation of SUMO–PML conjugates and the disruption of PML bodies[94]. Because PML sumoylation seems to regulate p53 activity, the virus-induced destruction of nuclear bodies and desumoylation of PML could be a viral strategy to overcome the negative effect of p53 on cell proliferation and apoptosis. Indeed, expression of BZLF1 can inactivate p53 function[95].

Intriguingly, viral and bacterial cysteine proteases have been identified that have sequence similarity to ubiquitin-like protein-processing enzymes (ULPs)[21,96]. One of these proteins, the *Yersinia* protein YopJ, seems indeed to interfere with the cellular SUMO-conjugation system[97]. So pathogens might have evolved a strategy to alter the sumoylation activity of the host for their own benefits.

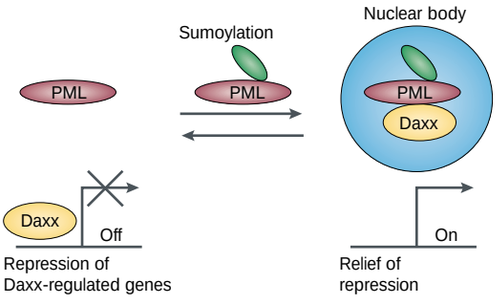

Figure 4 | **Sumoylation of PML modulates Daxx-mediated transcriptional repression.** Sumoylation of PML recruits Daxx to nuclear bodies and removes it from its target genes, thereby relieving Daxx-mediated repression of these genes.

as one of the most important substrates for SUMO conjugation[64]. SUMO and Ttk69 proteins localize together on POLYTENE CHROMOSOMES, indicating that the SUMO-conjugated Ttk69 species is present at sites of Ttk69 action *in vivo*. It is unknown how SUMO regulates transcriptional processes mechanistically. However, with respect to the role of SUMO in complex formation, it is tempting to speculate that sumoylation can modulate the interaction of transcription factors with transcriptional coregulators.

...or an inhibitor of ubiquitylation? Studies on the NF-κB inhibitor IκBα and the ubiquitin-ligase Mdm2 have uncovered an interesting functional link between the ubiquitylation and sumoylation systems[65,66]. The NF-κB transcription factor is kept inactive in the cytosol by binding to its inhibitor IκBα. Upon stimulation with effectors such as tumour necrosis factor (TNF), IκBα is phosphorylated, ubiquitylated and subsequently degraded by proteasomes[67]. This liberates NF-κB from its inhibitor, thereby allowing the transcription factor to enter the nucleus and activate its target genes. Intriguingly, SUMO can compete with ubiquitin on IκBα, as both modifiers target the same lysine residue in IκBα. The SUMO-modified pool of IκBα is protected from TNF-induced degradation, and sumoylation of IκBα inhibits NF-κB function.

The NF-κB/IκBα system is an evolutionarily conserved pathway; its counterpart in *Drosophila* is the Dorsal/Cactus system, in which Dorsal is the vertebrate homologue of NF-κB and Cactus corresponds to IκBα. Surprisingly, however, it is Dorsal — not Cactus — that undergoes sumoylation[68]. In this case, sumoylation facilitates nuclear import of Dorsal and thus, in contrast to the mammalian system, activates its function. Hence, it seems that SUMO can regulate similar pathways in different organisms, but the target proteins might not necessarily be the same.

Recent data on Mdm2 provide evidence that SUMO might have a more general role in protein stabilization. Mdm2 is a RING-finger protein that serves as an E3-type ubiquitin ligase for p53 and for itself[69,70]. Interestingly, the stability of Mdm2 itself is also regulated by the ubiquitin–proteasome system and it has been proposed that SUMO interferes with the ubiquitylation of Mdm2 (REF. 66). Similarly to what was observed for IκBα, SUMO and ubiquitin were reported to act on the same lysine residue within the RING finger of Mdm2. Hence, sumoylation of Mdm2 would prevent ubiquitylation and subsequent degradation. In normal cells, most Mdm2 has mobility corresponding to a protein of 90 kDa, which could represent a SUMO–Mdm2 conjugate. Upon DNA damage, however, Mdm2 is presumed to be de-sumoylated and subsequently ubiquitylated and degraded. As a result of decreasing cellular Mdm2 levels, p53 is stabilized. Although this model seems appealing, a note of caution has been raised recently[71]. In particular, it is unclear why bacterially expressed, unsumoylated Mdm2 comigrates in gels (90 kDa) with the presumed SUMO–Mdm2 conjugate. Furthermore, the 90 kDa Mdm2 species is insensitive to ULP-mediated de-sumoylation *in vitro* (Müller, S. and Dejean, A., unpublished observations). Mass spectrometric analysis of the 90-kDa species is expected to settle these issues and to define the nature of this species.

What does genetics tell us?

In the budding yeast *S. cerevisiae*, sumoylation is essential for viability. By contrast, SUMO from the yeast *Schizosaccharomyces pombe* is not essential for viability, but mutants defective in this pathway show strong growth defects[72]. Interestingly, temperature-sensitive mutants in the *S. cerevisiae* genes for the SUMO-activating enzyme (*uba2-ts*), SUMO-conjugating enzyme (*ubc9-ts*) and the de-sumoylation enzyme Ulp1 (*ulp1-ts*) show strong cell-cycle defects[12,21,73]. They predominately arrest at the G2/M boundary of the cell division cycle as large-budded cells with replicated DNA and a short MITOTIC SPINDLE indicating that sumoylation of one or more targets in yeast is essential for cell-cycle progression at this phase. Mutants deficient in Ulp2 are viable but they show various phenotypic abnormalities, such as aberrant cell-cycle progression, hypersensitivity to DNA damage and chromosome mis-segregation[22]. *ULP2* messenger RNA levels are upregulated during early sporulation, pointing to a role for de-conjugation of certain substrates during yeast meiosis.

The main targets of SUMO in *S. cerevisiae* are the SEPTINS Cdc3, Cdc11 and Sep7, which form a 10-nm filamentous ring that encircles the yeast bud neck[74,75]. Proper assembly of the septin ring is monitored by an unknown mechanism at the morphogenesis checkpoint, which acts at the G2/M phase boundary of the cell cycle. Sumoylation of the septins occurs during mitosis before anaphase and the modifications disappear abruptly at cytokinesis. Disappearance of this neck ring is disturbed in a yeast mutant strain in which all the SUMO-conjugation sites of the septins are changed to arginine residues, arguing that the dynamics of the neck ring require sumoylation. However, the G2/M arrest of mutants with deficiencies in the SUMO conjugation pathway (*uba2-ts*, *ubc9-ts*) cannot be explained by the absence of sumoylated septins, because mutants that express septins lacking the SUMO conjugation sites show no growth defects.

POLYTENE CHROMOSOME
A giant chromosome formed by many replications of the DNA. The replicated DNA molecules tightly align side-by-side in parallel register, creating a non-mitotic chromosome that is visible by light microscopy.

IκBα
Inhibitory subunit of the NF-κB transcription factor, which is phosphorylated, ubiquitylated and degraded in response to activating stimuli.

MITOTIC SPINDLE
A highly dynamic bipolar array of microtubules that forms during mitosis or meiosis and serves to move the duplicated chromosomes apart.

SEPTINS
Highly conserved protein family first identified in yeast and more recently found in a wide range of animal cells. They are thought to function primarily in the control of cytokinesis, where they form a 10-nm filamentous ring that encircles the yeast bud neck.

HIGH-COPY SUPPRESSOR
Gene that suppresses a phenotype when expressed at high copy number.

CENTROMERE
Region of a chromosome that is attached to the spindle during nuclear division.

MINICHROMOSOME
An extrachromosomal plasmid DNA that contains a chromosomal origin of replication.

SYNAPTONEMAL COMPLEX
Structure that holds paired chromosomes together during prophase I of meiosis and that promotes genetic recombination.

WERNER SYNDROME
A rare autosomal recessive disorder, characterized by the early development of various age-related diseases. The gene responsible for Werner syndrome (*WRN*) encodes a DNA helicase homologous to *Escherichia coli* RecQ.

BLOOM SYNDROME
A rare cancer-predisposing autosomal recessive disorder characterized by genomic instability, immunodeficiency and small stature. *BLM*, the gene mutated in Bloom syndrome, encodes a DNA helicase of the RecQ family.

A first hint of SUMO's importance for the maintenance of genomic integrity came from genetic studies in yeast. Yeast SUMO was originally identified as a HIGH-COPY SUPPRESSOR of mutations in Mif2 (REF. 76). The MIF2 protein is part of a centromeric multiprotein complex and is required for proper segregation of chromosomes and for integrity of the mitotic spindle[77]. The interaction of Ubc9 with CENTROMERE proteins from *S. cerevisiae* supports the idea that sumoylation could regulate centromeric proteins. In the yeast *S. pombe*, deletion of the gene for either SUMO (*pmt3*$^+$) or Ubc9 (*hus5*$^+$) leads to aberrant mitosis, increased sensitivity to DNA-damaging agents and high-frequency loss of MINICHROMOSOMES, indicating that Pmt3 is also involved in processes such as chromosome segregation and DNA-damage repair[78]. However, a molecular understanding of these phenotypes requires the identification of the SUMO substrates. A role for SUMO in DNA-damage repair and recombination is further supported by the finding that the human homologues of yeast Rad51 and Rad52, physically interact with both Ubc9 and SUMO in yeast two-hybrid assays[79,80]. In addition, Ubc9 localizes together with Rad51 in SYNAPTONEMAL COMPLEXES during meiosis[81]. Whether Rad51 and Rad52 are modified by SUMO is, at present, not known. The Rad51/Rad52 pathway is involved in DNA recombination and the repair of DNA double-stranded breaks. Intriguingly, overexpression of SUMO downregulates homologous recombination after DNA double-stranded breaks and reduces cellular resistance to ionizing radiation[82]. Additional known SUMO substrates with links to DNA repair are human DNA topoisomerase I and II, which both become sumoylated upon DNA damage[83,84] and the WERNER SYNDROME gene product, a DNA helicase of the RecQ family[85]. Furthermore, another member of the RecQ helicase family, the BLOOM SYNDROME protein (BLM), interacts with Ubc9 (REF. 85). Interestingly, some of the proteins mentioned above, such as Rad51 and Rad52, BLM and CENP-C (the human Mif2 homologue), are transiently associated with PML nuclear bodies[48,86–89]. However, conclusive data on whether PML nuclear bodies are implicated in the maintenance of genomic stability and repair are missing.

From substrates to function

Studies on SUMO have entered an exciting phase. An astonishing number of important cellular regulators and functions are now known to be controlled by sumoylation. It seems that the reversible nature of this modification is a crucial factor in these processes. The findings described above implicate SUMO in the stabilization of proteins and/or their localization to subcellular complexes. Whether SUMO targets the proteins to these complexes or whether conjugation occurs within these complexes is a key issue that has to be settled. The available data on the most intensely studied SUMO substrates, PML and RanGAP1, suggest that their sumoylation promotes specific protein–protein interactions or stabilizes preformed protein complexes. The reversible nature of the SUMO modification might be well suited to dynamically regulate these protein complexes. A different aspect of sumoylation became evident from the findings on IκBα and Mdm2, in which SUMO seems to counteract the function of ubiquitin, thereby blocking ubiquitin/proteasome-mediated proteolysis. Although theses findings are intriguing, SUMO clearly does not always function in this way. For example, the sumoylation and ubiquitylation sites within p53 seem to be different and sumoylation does not directly interfere with the ubiquitylation of p53 (REFS 53, 54). It seems possible that SUMO functions in different ways depending on its substrate. Could both suggested functions of SUMO be mechanistically linked (FIG. 5)? An attractive hypothesis is that proteins within large assemblies that are induced by sumoylation are more resistant to ubiquitin/proteasome-dependent degradation than their respective free counterparts. Consistent with this model is the observation that the abrogation of PML sumoylation by the Herpes simplex virus ICPO protein not only disassembles PML nuclear bodies, but subsequently induces the proteasome-dependent degradation of PML[90]. Given the pace of recent research on this enticing and rewarding field, it can be hoped that the near future will uncover more of SUMO's secrets.

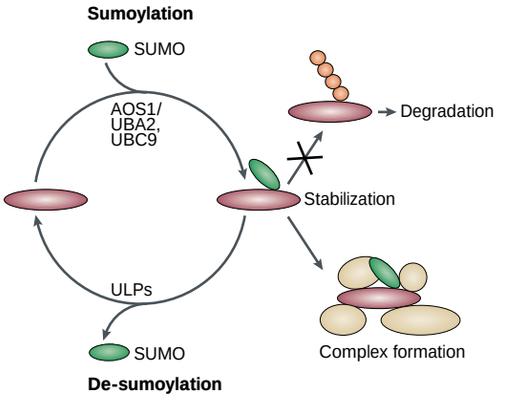

Figure 5 | **Reversible modification of proteins by SUMO and its consequences.** Sumoylation either prevents ubiquitylation followed by degradation or results in the formation of protein complexes. SUMO is depicted in green, ubiquitin in red. Sumoylation requires AOS1/UBA2 and UBC9 enzymes; de-sumoylation is catalysed by members of the ULP family.

Links

DATABASE LINKS ubiquitin | RUB1 | Apg8 | Apg12 | parkin | RAD23 | DSK2 | *SMT3* | SUMO-1 | SUMO-2 | SUMO-3 | RanGAP1 | AOS1 | UBA2 | UBA1 | UBC9 | UBC4 | UBC7 | ULP1 | ULP2 | SMT3IP1 | SENP-1 | SUSP-1 | Ran | RanBP2 | RNA1 | *semushi* | PML | p53 | Daxx | p73 | Sp100| HP1 | HIPK2 | TEL | c-Jun | androgen receptor | Tramtrack 69 | NF-κB | Mdm2 | IκBα | TNF | Dorsal | Cactus | RING finger | CDC3 | CDC11 | SEP7 | MIF2 | RAD51 | RAD52 | DNA topisomerase I | DNA topoisomerase II | RecQ | BLM | CENP-C
FURTHER INFORMATION Jentsch lab
ENCYCLOPEDIA OF LIFE SCIENCES Nuclear–cytoplasmic transport | Ubiquitin pathway

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