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SUMO Tasks in Chromatin Remodeling

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http://dx.doi.org/10.5772/55395

1. Introduction

Post-translational modifications implicate attachment of diverse molecules to proteins after translation. These modifications are essential for many biological processes as they are involved in their regulation. From relatively simple molecules to small polypetides are common covalent modifiers of proteins. Sumoylation consists in the post-translational modification of proteins by attachment of the small polypeptide SUMO (small ubiquitin-like modifier). This post-translational modification was identified two decades ago and has been very actively investigated to date. Sumoylation has consequences on protein structure and regulation. This modification controls many processes in the eukaryotic cell and is essential for viability of all the organisms studied so far.

From the discovery of ubiquitin in 1975, a number of ubiquitin-like proteins (UBLs) have been identified in eukaryotes and it has been shown that many of them are able to covalently attach to other proteins (reviewed in [1]). Several aspects are common to most UBLs: they are small polypeptides (less than 200 amino acids) capable of attaching to other macromolecules in a covalent way, present common structural features and use similar modification pathways. These characteristics strongly support duplication and diversification during evolution as the origin of the different pathways. Ubiquitin and UBLs are characterized by the presence of the ß-grasp fold, which also appears in ubiquitin-like domains of several other proteins of the ubiquitin system and in numerous non-related proteins (reviewed in [2]). The ß-grasp fold seems to have emerged in prokaryotes as a translation-related RNA-binding module, which diversified structurally and biochemically before to dramatically expand in eukaryotes [2]. Besides ubiquitin and SUMO, examples of UBLs are NEDD8, FUBI, FAT10, ISG15, UFM1, Atg8, Atg12 and Urm1 (reviewed in [1]).
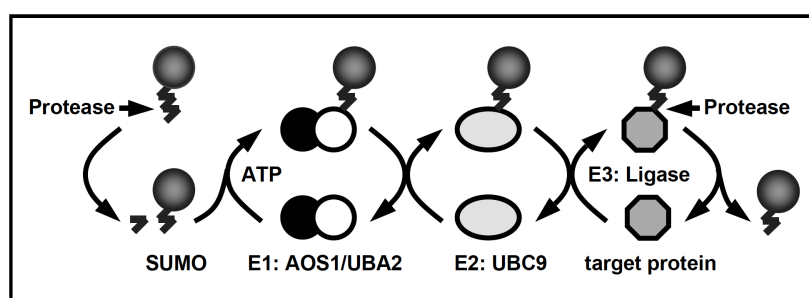
The first report of a protein being modified by SUMO occurred in the nineties and concerned the mammalian nuclear pore-associated GTPase activating protein RanGAP1 [3, 4]. Subse-

quently, more than a hundred proteins have been identified as SUMO substrates. Although similarities with ubiquitin are notable [5], SUMO plays many regulatory functions in the cell that significantly differ from the major role displayed by ubiquitin: labeling proteins to target them for proteasomal degradation [6]. A variety of consequences derived from protein sumoylation (new interaction surfaces, modulation of protein affinity and binding capacities to other molecules, modulation of protein activity, blocking of protein domains, steric hindrance, crosstalk or interference with other post-translational modifications) account for the many roles attributed to SUMO (reviewed in [7]). A major role of SUMO is associated with RanGAP1 and thereby with the nuclear pore complex. Thus, involvement of SUMO in nucleo-cytoplasmic transport of proteins has been well established [8]. SUMO has been also implicated in chromosome dynamics in mitosis and meiosis (condensation, cohesion, separation) and genome integrity, as many proteins involved in DNA replication, repair and recombination are modulated by SUMO modification (reviewed in [7]). Other roles attributed to SUMO are related to enzyme regulation, protein stability and cellular structure (reviewed in [9, 10]). However, the most prominent function of SUMO concerns transcriptional regulation, and specially transcription repression (reviewed in [11, 12]). The role of SUMO in transcription, in the context of chromatin structure and dynamics, is analyzed in this chapter.

2. The modification pathway

2.1. Enzymes involved

Modification by SUMO involves the ATP-dependent activation of mature SUMO (C terminus of SUMO needs to be excised by proteolysis) by the E1 enzyme, transfer to the E2 enzyme UBC9 and conjugation to the target protein, often mediated by a SUMO ligase or E3 (Figure 1 and Table 1) (reviewed in [9]). Maturation of the SUMO precursor, as well as removal of SUMO from targets is displayed by SUMO specific proteases.

Figure 1. The sumoylation pathway. Cleavage of the SUMO C terminus enables ATP-mediated activation and binding to the E1 enzyme to be transferred to the E2 conjugating enzyme UBC9, which mediates target modification with the concourse of an E3 SUMO ligase. Recycling of SUMO is performed by the same proteases involved in maturation.

SUMO E1 activity is performed by the SAE1/UBA2 heterodimer in human, in contrast to the ubiquitination pathway where the E1 activity is displayed by a monomeric enzyme. However, the SAE1 subunit is homologous to the N-terminal part of ubiquitin E1, while the UBA2 subunit

is homologous to its C terminus [13]. Both monomers work together and are not found separately [14]. E1 activation of mature SUMO involves ATP hydrolysis and formation of a thiolester bond between E1 and the C terminus of SUMO before being transferred to the E2. While several E2 have been described for ubiquitination, UBC9 is the only E2 known for sumoylation [15, 16]. Thus, UBC9 is the conjugating enzyme directly involved in attachment of SUMO to the different substrates. This second step of the sumoylation reaction involves the formation of a thiolester bond between SUMO and UBC9 upon transfer from the E1. The region surrounding the active site cysteine (C93 in mammals) in UBC9 is able to directly interact with sumoylation consensus sequence (see below) in target proteins [17-19].

Enzyme	Protein	Activity	References
E1 (activating)	AOS1/UBA2	ATP-mediated activation of SUMO	[13]
E2 (conjugating)	UBC9	SUMO conjugation to target	[15, 16]
E3 (ligase)	PIAS1-4	Facilitates transfer to target	[20, 21]
	RanBP2		[22]
	Polycomb-2 (Pc2)		[23]
	TOPORS		[24]
	Class IIa HDACs		[25]
	KAP-1		[26]
	RHES		[27]
	Krox20		[28]
protease	SENP1-3, 5-7	Maturation/recycling	[29-31]
	DeSI-1		[32]

Table 1. Enzymes involved in SUMO conjugation.

SUMO ligases are involved in facilitating the SUMO attachment to substrates (reviewed in [33]). To date, few ligases have been described for sumoylation, in contrast to ubiquitination, where lots of them are known to play an essential role and mediate substrate specificity. In fact, SUMO ligases were undervalued at the beginning, since certain substrates are sumoylated in vitro, provided that E1 and E2 are present at the adequate concentrations. Since UBC9 is able to directly interact with sumoylation consensus sequence in substrates, it is able to render sumoylation in the absence of a ligase. However, a number of proteins, which augmented the efficiency of SUMO conjugation, were identified. The list of SUMO ligases progressively increases and essential roles for these have been described in vivo (see [34]). Although mechanisms of action of SUMO ligases have not been completely elucidated, it is obvious that many ligases facilitate transfer of SUMO by bringing together SUMO-loaded UBC9 and the target protein. Thus, similar to the RING domain-containing E3 ligases involved in ubiquitination, SUMO ligases do not establish a covalent bond with SUMO. In this context, a SUMO ligase should normally i) interact with the substrate, ii) interact with UBC9, iii) facilitate SUMO

transfer to the substrate. Ligases of the PIAS family (PIAS1 to 4) have been extensively studied [35]. They present a type of RING finger domain, the SP-RING (Siz/PIAS RING), for UBC9 interaction, although Ubc9 binding to a PHD domain in plant PIAS proteins has been also described [36]. Other SUMO ligases described so far are RanBP2 [22], the Polycomb-2 (Pc2) protein [23], class IIa histone deacetylases (HDACs) [25], topoisomerase I-binding RING finger protein (TOPORS) [24], the PHD containing protein KAP-1 [26], Ras homologue enriched in striatum (RHES) [27] and the transcription factor Krox20 [28]. In contrast to most ubiquitin ligases, SUMO ligases may display significant promiscuity, as many of them enhance sumoylation of a variety of substrates.

SUMO proteases are involved in maturation of the SUMO precursor by exposing two glycine residues at the C terminus for binding to E1 [37]. In addition, they are also involved in SUMO recycling by excising the SUMO moiety from substrates. Yeast Ulp1p was the first SUMO protease identified [29]. Sequence analysis revealed that it corresponded to a protease of the C48 cysteine group, not related to deubiquitylating enzymes but similar to adenovirus proteases. Mammalian SUMO proteases are represented by the SENP (sentrin-specific protease) family. It comprises six members, SENP1 to 3, and SENP5 to 7 [38]. A seventh member, initially identified as SENP4, resulted to actually correspond to SENP3. Besides SENP1 to 7, an additional family member has been reported, SENP8. However, this protease does not act on SUMO, but on another UBL, NEDD8 [39, 40]. Very recently, a new type of SUMO protease has been described, the desumoylating isopeptidase 1 (DeSI-1) [32]. The different SUMO proteases show diverse cellular localization and different specificities for the various SUMO molecules and substrates (reviewed in [38]).

2.2. SUMO molecules

Four different SUMO molecules have been described in mammals: SUMO1 to 4. SUMO1 has been implicated in regulation of many processes, while SUMO2 and SUMO3 are highly related with the response to stress. Consequently, a significant pool of free SUMO2 and SUMO3 is detected in the cell, which is rapidly mobilized after exposure to a variety of stress conditions. In contrast, most of SUMO1 appears conjugated to proteins [41]. SUMO2 and SUMO3 are usually referred as SUMO2/3, as they share 97% identity and antibodies hardly differentiate the two forms. By contrast, SUMO1 only shares about 50% identity with SUMO2/3. Despite the low similarity showed between ubiquitin and SUMO (about 18% identity with SUMO1), structurally they are quite similar, excepting the N-terminal region of SUMO not present in ubiquitin [42]. A remarkable difference between SUMO1 and SUMO2/3 is the ability of this last to form poly-SUMO chains in vitro as well as in vivo, due to the presence of a sumoylation consensus sequence in the molecule [43]. SUMO4 is the last SUMO molecule identified. It shows a restricted expression pattern [44] and several data bring into question its capacity to be conjugated to proteins [45]. However, a polymorphism found in human SUMO4 correlates with type 1 diabetes [46]. The different SUMO molecules share a common modification pathway and the existence of functional redundancy has been suggested. However, specific modification by the different SUMO paralogs has been implicated in the regulation of a variety

of processes. Thus, the modification pathway is able to differentially conjugate the various SUMO molecules depending on the substrate or the regulatory process [47].

2.3. Sumoylation consensus motifs and SUMO interacting motifs

Covalent attachment of SUMO occurs through the ε-amino group of a lysine residue in target proteins. In many cases the Lys (K) residue is the core of the consensus sequence ΨKxE, being Ψ a large hydrophobic residue and x any amino acid. Extended consensus (phosphorylation-dependent SUMO motif (PDSM) and negatively charged residues-dependent SUMO motif (NDSM)) and variations have been described as well (Table 2) (reviewed in [34]). However, sumoylation also occurs at non-consensus sequences. As mentioned above, the consensus sequence is directly contacted by UBC9. Thus, it is possible that when sumoylation occurs at non-consensus sequences, certain amino acid residues, otherwise dispersed in the primary structure of the target protein, bring together in the three-dimensional structure to mimic a consensus-like environment. It is worth noting that conversely, sumoylation consensus sequences in a protein are not always substrate for SUMO attachment, indicating that additional structural features regulate and enable modification by SUMO. Besides covalent attachement of SUMO, many proteins can associate with SUMO in a different way involving a non-covalent interaction (reviewed in [48]). This occurs through SUMO interacting motifs (SIMs) in proteins. SIMs are usually characterized by the presence of a short hydrophobic region surrounded by negatively charged residues (Table 2) [49]. The non-covalent interaction of proteins with SUMO has been revealed essential in the regulation of several processes. In a variety of cases function of the system relies in the combinatorial occurrence of sumoylation sites and SIMs in a given protein or in different subunits of a complex, which determines its macromolecular architecture (Figure 2 and see below). This situation is exemplified by the promyelocytic leukaemia protein (PML), in which combination of sumoylation sites and SIMs dictates the formation of PML nuclear bodies and the recruitment of additional proteins [48].

2.4. Regulation of sumoylation

Despite that certain SUMO targets appear constitutively sumoylated, it is obvious that sumoylation, as a signaling pathway needs to be regulated. A striking feature of SUMO modification consists in the so-called "SUMO enigma" [9]. It has been observed that many SUMO targets are difficult to detect at the sumoylated state, but mutation of the acceptor lysine has severe consequences in the process involved. In other words, at the steady state, only a low proportion of the whole pool of a given target appears sumoylated, although sumoylation results essential for function of the target. Thus, sumoylation has been suggested to be a highly dynamic and transient modification that permanently marks targets for specific fates even though the SUMO moiety has been removed [9]. This can be explained by viewing sumoylation as a temporal facilitator for the establishment of protein interactions, other protein modifications, or sub-cellular localization (Figure 2).

Sumoylation can be regulated at different levels (reviewed in [34, 50]). First level of regulation in SUMO modification relies in the nature of target proteins, as target sequence, structural features, and other protein modifications affect attachment of SUMO. The other way to

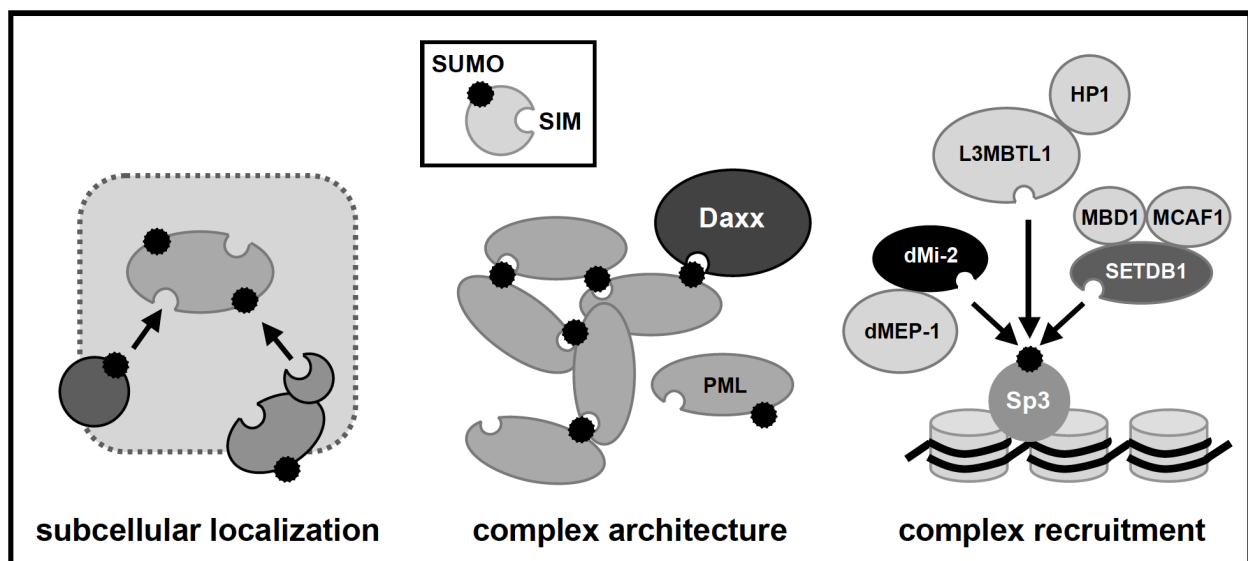| SUMO binding	Type		Sequence
Sumoylation site	Consensus		ΨKxE
	Extended consensus	PDSM	ΨKxExxSP
		NDSM	ΨKxExx[D/E]$_n$
	Iverted consensus		ExKΨ
	Phosphorylated Ser		ΨKxS
SIM	ZNF198		DDDDDDD VVFI
	PIAS1		VEVI DLTI DSSSDEEEEE
	SP100		IIVI SSEDSEGSTDVD
	PML		EE R VVVI SSSEDSD
	RanBP2		SDSPSDDD VLIV
	CoREST1		EESEDELEE ANGNNP IDIEV

Table 2. Sumoylation sites and SUMO interacting motifs (SIMs). Ψ represents a large hydrophobic residue. Sumoylated Lys (K) is frequently close to a hydrophobic residue and to negatively charged environment, either acidic residues Asp/Glu (D/E) or phosphorylation sites (SP). SIMs usually consist in a stretch of 4 amino acids, containing at least 3 hydrophobic residues, close to an acidic region (Asp/Glu) (D/E) or putative phosphorylation sites (Ser/Thr) (S/T). Examples of SIMs with acidic/phosphorylation region N-terminal to the hydrophobic core (ZNF198), C-terminal (with spacer (PIAS1), without spacer (SP100)), at both sides (PML), SUMO1 specific (RanBP2) and SUMO2/3 specific (CoREST1), are shown.

regulate sumoylation depends on the modification pathway. Availability of the different SUMO paralogs when sumoylation is required, or acting on the E1 and E2 enzymes, represents a global way to regulate sumoylation. For instance, stress conditions normally leads to SUMO2/3 conjugation, as SUMO2/3 is freely available in the cell [41]. On the other hand, it has been shown that expression of the Gam1 protein by the CELO adenovirus leads to E1 and E2 degradation and thereby to inhibition of sumoylation [51]. Finally, a more selective way of regulating sumoylation is given through the activity of SUMO ligases and proteases. Thus, localization or spatiotemporal regulation of the expression of these proteins has consequences in target sumoylation.

3. SUMO in transcription

3.1. Transcription repression

It is of significance that the sumoylation consensus sequence, before being established to be the site for SUMO attachment, was initially identified as a negative regulatory sequence in several transcription factors [52]. This scenario is exemplified by the transcription factor Elk-1 (Ets (E twenty-six)-like kinase 1), where a repressive domain, the R motif, was identified as an acceptor region for SUMO attachment [53]. Targeting SUMO or the SUMO conjugation enzyme UBC9 to promoters through a Gal4-based system efficiently represses transcription [54-56],

Figure 2. Sumoylation and SIMs are involved in complex architecture and function. Schematic representation of some examples for SUMO-SIM interactions involved in recruiting proteins to a particular subcellular localization, in the architecture of PML aggregates and association to Daxx, and in recruitment of different repressor complexes to the chromatin through sumoylated Sp3 for transcription repression.

indicating that sumoylation mainly associates with transcription repression. Examples from different organisms have argued in favor of such a role. A characteristic of silenced genes is that they correlate with low levels of histone acetylation, while active genes usually display high histone acetylation. It has been described in yeast that temperature-sensitive mutation in Ubc9 leads to an increase in global histone acetylation [57]. In addition, in fission yeast, it has been shown that SUMO is required for the maintenance of heterochromatin stability [58]. Early evidence of the involvement of the SUMO pathway in maintenance of the heterochromatin came from Drosophila, as a PIAS mutant was identified as a suppressor of position effect variegation, that is, as a mutant releasing heterochromatin-induced gene silencing [59]. A mechanism that clearly account for the repressive role of SUMO is explained by the ability of SUMO to recruit histone deacetylases [56]. For many transcription factors sumoylation has been linked to transcription repression. Additional examples to Elk-1 are NAB proteins [28], c-Jun [60], p53 [61], IκBα [62], C/EBP [63], Sp3 [64] and MEF2 [65]. It is worth noting that in many cases sumoylation turns activators into repressors, as it is the case of p300 and CREB binding protein (CBP) [66, 67]. However, beyond SUMO modification of transcription factors, SUMO association with architecture and function of chromatin-associated repressor complexes is recently getting increased importance. This has been reviewed in [68, 69] and is described below.

3.2. Transcription activation

Despite the clear association of SUMO with gene repression, several reports illustrate the involvement of sumoylation in transcription activation. Examples of transcription factors whose activity is stimulated by SUMO are TCF4 [70], GATA4 [71], Pax6 [72], p45 [73], Smad4

[74], Oct4 [75], p53 [76], myocardin [77], PEA3 [78], NFAT1 [79] and HSF1 and 2 [80, 81]. Intriguingly, p53 has been reported both to be activated and repressed by SUMO [76, 82]. Since sumoylation may compete other post-translational modifications, a mechanism proposed for SUMO-mediated activation of transcription consists in avoiding degradation, and thereby in stabilization, of the transcription factor, as it has been proposed for Oct4 [83]. Otherwise, SUMO modification may interfere with association of repressors with the transcription factor, as occurs for Ikaros, whose sumoylation avoid interaction with histone deacetylase complexes [84]. Recently, two publications have brought into consideration the general assumption that SUMO globally associates with transcription repression. It has been reported in yeast that SUMO is detected at all the constitutively transcribed genes tested and in inducible genes upon activation [85]. However, Ubc9 inactivation results in increased transcription of inducible genes, although sumoylation at promoters is reduced, suggesting a role for SUMO in the silencing of inducible genes. In sum, authors conclude that while SUMO associates with repression in some contexts, other properties of SUMO come into play under normal constitutive transcription [85]. More recently, a study performed in HeLa cells has revealed that from G1 to S phase of the cell cycle SUMO1 marks chromatin at the proximal promoter region on many of the most active housekeeping genes [86]. SUMO1 depletion results in reduced expression of these genes. However, this occurs for half of the active genes and the nature of the sumoylated proteins at the promoters remains unknown [86]. Taken together, all these data indicate that although SUMO may intrinsically associate with transcription repression, many other general processes, including constitutive transcription, may also depend on sumoylation, structurally or as a signaling pathway.

4. Histone modification and chromatin remodeling

4.1. Histone sumoylation

Regarding histone modification, sumoylation has been implicated in both, direct modification of histones and deposition/recognition of other histone marks, such as acetylation and methylation. Histone sumoylation has been demonstrated in both yeast and mammal cells [55, 57]. All core histones and the H2A.Z variant have been shown to be sumoylated in yeast [57, 87], while work on mammal cells has been centered on histone H4 [55]. The N-terminal tail of canonical histones is the target for sumoylation, indicating that sumoylation may interplay with other histone modifications at this region, like acetylation, methylation and phosphorylation. Interfering with the sumoylation pathway significantly reduces the level of histone sumoylation in yeast [57]. Histone sumoylation has been associated with transcription repression. Indeed, mutation of sumoylation sites in histone H2B in yeast leads to increased basal expression of several non-induced genes [57]. A more specific role in Rad51-labeling of persistent DNA double strand breaks has been attributed to sumoylation of the histone variant H2A.Z in yeast [87]. However, which is the real impact of histone sumoylation in transcription in vivo and whether it is a common feature all along the genome need to be clarified.

4.2. Involvement of SUMO in recognition of histone modifications

As explained before, sumoylation of histone tails may affect the way in which different proteins recognize other histone modifications. Conversely, sumoylation of a chromatin-associated factor may modulate its capacity to recognize a specific histone modification. For instance, it has been reported that sumoylation of the bromodomain GTE3 protein, a BET (bromodomain and extra terminal domain) family member, interferes with the capacity of this protein to associate with acetylated histone tails [36]. A surprising link between the sumoylation pathway and recognition of histone modifications is illustrated by a recent and intriguing report describing the capacity of the PHD domain of plant PIAS proteins to directly recognize histone modifications such as methylated Lys4 and Arg2 on histone H3 (methyl-H3K4 and methyl-H3R2) [88].

Polycomb group (PcG) proteins are involved in regulation of gene transcription and chromatin structure especially during development. These transcriptional repressors regulate lineage choice during development and differentiation by establishing long-term heritable gene silencing of relevant genes, for instance *Hox* genes. Thus, they are tightly linked to stem cell biology and cancer [89]. Two main complexes assembling PcG proteins have been described [90]. The polycomb repressive complex 2 (PRC2) contains the histone methyl transferase Enhancer of Zeste (EZH2) and is involved in methylation of H3K27. The PRC1 complex contains the Polycomb protein, which is involved in recognition of the repressive mark trimethyl-H3K27 through a chromodomain. Recruitment of PRC1 to the chromatin results in ubiquitination of histone H2A. Hence, coordinated action of both complexes is involved in the establishment of a compact chromatin structure, which results in gene silencing. One of the mammalian orthologs of Drosophila Polycomb is Polycomb-2 (Pc2), which has been shown to display SUMO ligase activity, as previously mentioned [23]. Interestingly, two SIMs have been described in Pc2, one of them has been shown to be relevant for the several functions attributed to Pc2 [91]. Among the SUMO substrates identified for Pc2 are the kinase HIPK2 and the corepressor CtBP1 (see also below), sumoylation of which results in enhanced transcription repression [92-94]. CtBP has been shown to colocalize with Pc2 in nuclear foci called PcG bodies, which contain several PcG proteins [95]. Other Pc2 substrates for sumoylation are ZEB2, DNMT3A and centrin-2 [96, 97]. It has been recently reported that Pc2 mediates sumoylation and recruitment of BMI1 at sites of DNA lesions, linking Pc2 ligase activity with the DNA damage response [98]. Several polycomb subunits have been shown to be sumoylated, for instance SUZ12, EZH2 and YY1, although Pc2 has not been involved in the process [99, 100]. A clear role of sumoylation in PcG proteins-mediated repression came from studies in *C. elegans*. The SOP-2 protein is related to Drosophila and vertebrate PRC1-associated PcG proteins Polyhomeotic and Sex combs on midleg (Scm). It has been shown that sumoylation of SOP-2 is required for repression of *Hox* genes in *C. elegans* [101]. Indeed, impaired sumoylation leads to ectopic *Hox* gene expression and homeotic transformations, resulting in a phenotype similar to that provoked by *sop-2* mutations. Additional evidence of SUMO involvement in PcG-mediated repression in vertebrates has been more recently reported. It was previously shown that Pc2 is a target of SUMO [23]. Later, Kang et al demonstrated that sumoylated Pc2 is a target for the SUMO protease SENP2 [102]. In *Senp2* knockout mice,

sumoylated Pc2 accumulates, resulting in increased occupancy at promoters of PcG target genes, such as *Gata4* and *Gata6*. As a result, expression of these genes is reduced during development, which leads to embryonic heart defects among other disorders [102]. Chromatin occupancy by PRC2 subunits and levels of trimethyl-H3K27 seem not to be affected, suggesting that Pc2 sumoylation has a role in recognition of H3K27 methylation, which is released by SENP2.

4.3. SUMO-mediated regulation of histone modifications

As previously mentioned, the major impact of sumoylation on histone modification is linked to the role of SUMO in the architecture and function of several chromatin-associated complexes involved in histone modification. Sumoylation by itself may condition the way other histone marks are deposited. However, it has been unambiguously demonstrated that sumoylation is essential for function of a variety of complexes implicated in histone modification, which mostly associate with transcription repression [68, 69]. It has been previously indicated that SUMO is required for the maintenance of constitutive heterochromatin in fission yeast [58]. However, increased evidence of SUMO involvement in the establishment of heterochromatin-like structures in euchromatin loci (facultative heterochromatin) has emerged during the last years. Facultative heterochromatin, besides displaying significant DNA methylation, is characterized by low levels of histone acetylation and histone H3 methylated at Lys4 (H3K4), and high levels of histone H3 methylated at Lys27 (H3K27) and Lys9 (H3K9, di- or tri-methylated), and histone H4 methylated at Lys20 (H4K20, mono-, di- or tri-methylated) [103]. Some of the complexes involved in the establishment of these marks are compiled in Table 3 and described below.

4.3.1. Histone methylation

The histone methyltransferase SETDB1 is involved in tri-methylation of H3K9, a repressive histone mark. The methyl CpG binding protein MBD1 and MCAF1 associate to SETDB1 in a complex, linking DNA methylation to histone methylation. This complex is recruited to the KAP-1 (KRAB associated protein-1) corepressor in a SUMO-dependent manner [26]. In its turn, sumoylated KAP-1 recruits the SETDB1 complex to the chromatin through the zinc finger protein KRAB. This is mediated by a SIM in SETDB1 [26]. In addition, another SIM has been reported in MCAF1, and both MCAF1 and MBD1 are sumoylated [104, 105]. Interestingly, a PHD domain in KAP-1 displays an E3 ligase activity, which promotes intramolecular sumoylation of the adjacent bromodomain [26]. The SETDB1 complex, as explained below, is also recruited to the transcription factor Sp3 in a SUMO dependent manner for transcription repression [106].

Recently, the SUMO ligase PIAS1 has been involved in maintaining an epigenetic repressive state, as studied at the *Foxp3* locus, that restricts differentiation of natural occurring thymus-derived regulatory T cells [107]. Knocking down of *PIAS1* leads to reduced DNA methylation and loss of the repressive mark methyl-H3K9 on the *Foxp3* promoter. A prominent role of PIAS1 in recruitment and association to the DNA methyltransferases DNMT3A and DNMT3B

is also reported. In correlation with loss of H3K9 methylation, HP1γ disappears from the *Foxp3* promoter in the absence of PIAS1 [107].

Complex (subunits)	Activity	Recruiting factor
LSD1/CoREST (LSD1, CoREST, BHC80, HDAC1/2, BRAF35, ZEB1, ZNF217/198)	H3K4 demethylation (LSD1) Histone deacetylation (HDAC1/2)	CtBP1
NurD (CHD3/4, HDAC1/2, RbAp46/48, MTA1/2, MBD3/2)	Nucleosome remodeling (CHD3) Histone deacetylation (HDAC1)	KAP-1
SETDB1 (SETDB1, MBD1, MCAF1)	H3K9 tri-methylation (SETDB1)	KAP-1 Sp3
L3MBTL1 (L3MBTL1, HP1)	Methyl-histone recognition (L3MBTL1)	Sp3
dMEC (dMi-2, dMEP-1)	Nucleosome remodeling (dMi-2)	Sp3
PCR2 (EZH2, EED, SUZ12, RbAp46/48)	H3K27 methylation (EZH2)	various
PCR1 (Pc2, PHC, RNF1/2, SCMH)	trimethyl-H3K27 recognition (Pc2)	–

Table 3. SUMO associated repressor complexes. Table summarizes some repressor complexes whose function depends on sumoylation. Examples of different transcription factors involved in recruitment of these complexes are also shown.

4.3.2. Histone demethylation

The histone demethylase LSD1 mediates gene repression by removing methyl groups from mono- or di-methyl-H3K4, which are marks of active transcription [108]. LSD1 works in a corepressor complex together with HDACs and CoREST1 [109, 110]. It has been shown that the LSD1/CoREST complex mediates SUMO-dependent repression of neuronal-specific genes, such as *SCN1A* and *SCN3A*, in non-neuronal cells [111]. Recruitment to the chromatin and repression depends on SUMO2/3 and is mediated by a specific SIM in CoREST. SUMO deconjugation by the SUMO protease SENP3 provokes increased levels of di-methyl-H3K4 and acetyl-H3, which leads to gene activation. Different subunits of the LSD1/CoREST complex have been shown to be sumoylated and/or to contain SIMs (reviewed in [68]). It has been recently shown that sumoylation of the LSD1/CoREST complex subunit BRAF35 controls neuronal differentiation [112]. Overexpression of BRAF35, but not of a sumoylation mutant, strongly impairs neuronal differentiation promoted by neurogenic factors in the vertebrate neural tube. Interestingly, iBRAF, a paralogue of BRAF35 ocasionally associated to the LSD1/CoREST complex, is not sumoylated but is able to dimerize with BRAF35, inhibiting BRAF35 sumoylation and binding to the LSD1/CoREST complex. The LSD1/CoREST complex usually

associates with the corepressor CtBP (C-terminal binding protein), which in turn is recruited to the chromatin by a variety of transcription factors [113]. Two CtBPs have been reported in vertebrates, CtBP1 and CtBP2. CtBP1 mediates repression by recruiting a number of repression factors that in addition to LSD1 and HDACs, includes the H3K9 histone methyl-transferase G9a. CtBP1-mediated repression depends on sumoylation [92]. Besides direct interaction of CtBP1 with UBC9, CtBP1 sumoylation is also determined by the SUMO ligases PIAS1, PIAS2 and Pc2 [23, 92, 114]. One of the transcription factors recruiting CtBP1 to the chromatin is the Krüpel-like zinc finger DNA-binding repressor ZEB1, which is also a target for sumoylation [115]. Attachment of SUMO to ZEB1 is required for this factor to display full repression activity [94]. Another zinc finger protein that has been associated with the LSD1/CoREST complex is ZNF198. This factor is both able to be sumoylated and to non-covalently interact with SUMO through a SIM [116-118]. Altogether, these data indicate that SUMO is involved on several functional aspects of the LSD1/CoREST complex: it mediates recruitment of the complex to the chromatin, but also is involved in the architecture of the complex, as different subunits associate to the complex in a sumoylation/SIM-dependent manner.

4.3.3. Histone deacetylation

It has been indicated that repression activity of the LSD1/CoREST complex is in part displayed through HDACs. Indeed, HDAC1 and HDAC2 are components of the LSD1/CoREST complex [110]. Another complex involved in HDAC recruitment to the chromatin is the NuRD (nucleosome remodeling and deacetylation) complex [119]. The core component of the mammalian NuRD complex is the ATP-dependent nucleosome remodeling enzyme CHD3. In addition, this complex includes one or two type I HDACs, histone binding proteins RbAp46 and RbAp48, a methylated DNA-binding protein (MBD2 or MBD3), and members of the MTA and p66 families of proteins [119]. A screening in Drosophila cell cultures identified the CHD3 homologue dMi-2, as a factor required for SUMO dependent repression by Sp3 [64]. dMi-2/CHD3 both sumoylates and is able to interact with SUMO-modified transcription factors through a SIM [26, 64]. Thus, CHD3 also interacts with sumoylated KAP-1 [26]. However, it has been demonstrated that phosphorylation of Ser824 in the C terminus of KAP-1, directly impairs interaction between the CHD3 SIM and the SUMO molecule attached to KAP-1 [120]. Therefore, KAP-1 sumoylation is not affected, but recognition of SUMO by the SIM in CHD3. KAP-1 phosphorylation has a role in double-strand break repair, as displaces the chromatin barrier imposed by CHD3-dependent nucleosome-remodeling activity. Additional components of the NuRD complex have been shown to be sumoylated and/or to contain SIMs: MTA1/2, HDAC1, RbAp48 and p66 [111, 121-123]. Interestingly, phenotype of certain vulval mutants in *C. elegans*, which associate with genes coding for NuRD components [124], is quite similar to that of *SUMO* and *UBC9* mutants [125], indicating that function of the NuRD complex is linked to sumoylation.

SUMO directly associates with HDACs in a variety of ways. As previously indicated a well-established link between SUMO and HDACs is illustrated by the SIM-mediated recruitment of HDACs to sumoylated proteins [56, 121]. It was first demonstrated for HDAC2 recruitment to sumoylated Elk-1 [56], and subsequently for HDACs 1, 3, 4, 5 and 6, and

class III SIRT1 deacetylases to several factors. Sumoylation of the coactivator p300 mediates recruitment of class II HDAC6 and class III SIRT1 deacetylases [66, 126]. HDAC1 recruitment to sumoylated Groucho, p68 and reptin has also been described [121, 127, 128]. Moreover, a SUMO-histone H4 fusion has been shown to precipitate HDAC1 [55]. Despite these data, it is not clear at present whether SUMO-dependent recruitment of HDACs involves direct binding of HDAC to SUMO or whether HDACs associate through cofactors recruited in a SUMO-dependent manner, as indicated for the LSD1/CoREST and NuRD complexes. Another example of SUMO-dependent recruitment of HDAC is depicted by the Daxx-mediated recruitment of HDAC2 to sumoylated CBP [67]. In this context, it is worth noting that in a variety of cases, HDAC recruitment does not account for full repression activity mediated by SUMO, as inhibition of HDACs does not relieve SUMO-dependent repression as expected. For instance, it has been shown in a reporter system that repression mediated by a Gal4-SUMO fusion is not sensitive to HDAC inhibition [56], as also occurs for SUMO-dependent Sp3-mediated repression [64, 129]. Despite HDAC2 recruitment by sumoylated Elk-1, HDAC2 knockdown only partially alleviates SUMO-dependent Elk-1-mediated repression [56]. Therefore, histone deacetylases are recruited in a SUMO-dependent manner through repressor complexes, together with additional repressor components, to account for full repression activity of the complex. Conversely, HDAC displacement by target sumoylation has been less reported, but examples have been described. Thus, sumoylation of the Prospero-related homeobox 1 (Prox1) and the de novo DNA methyltransferase DNMT3A disrupts association to HDAC3 and HDAC1/2, respectively [130, 131]. On the other hand, HDACs have also been shown to be substrates for SUMO, which regulates HDAC activity. Then, mutation of the sumoylation sites in HDAC1 has been shown to dramatically reduce its repression activity in a reporter assay [122]. It has been reported that the protease SENP1 is able to remove SUMO from sumoylated HDAC1, which leads to enhanced transcription activity by the androgen receptor [132]. Interestingly, the viral protein Gam1 interferes with HDAC1 sumoylation [133]. The RanBP2 ligase has been demonstrated to promote sumoylation of HDAC4 [134], and a relevant role for SUMO chain formation on HDAC4 has been attributed to the non-covalent interaction between SUMO and UBC9 [135]. Paradoxically, while HDAC1 sumoylation seems to be essential for its repression activity [122], SUMO attachment to HDAC1 impairs association to the CoREST repressor [116]. As previously mentioned, class IIa HDACs have been reported as SUMO ligases. HDAC4 and other class IIa HDACs promote SUMO2/3 attachment to the myocyte enhancer factor 2 family members MEF2D and MEF2C, which leads to repression of target genes [25]. Conversely, ligase activity is inhibited by HDAC4 sumoylation. HDAC4 ligase activity has been also demonstrated on liver X receptors sumoylation by SUMO2/3 [136] and on HIC1 sumoylation by SUMO1 [137], while enhanced sumoylation of PML protein has been attributed to HDAC7 [138].

4.4. Multiple complexes contribute to SUMO-dependent Sp3-mediated repression

Sp3 belongs to the specificity protein (Sp) family of transcription factors, which regulate multiple genes involved in housekeeping, development and cell cycle. Sp3 is expressed ubiquitously and can act either as an activator or a repressor depending on the promoter

context [106, 139]. Sp3-mediated repression depends on Sp3 sumoylation, and as previously indicated, this repression activity is not affected by HDAC inhibitors [129, 140].

A genome-wide RNAi screen in Drosophila cell cultures revealed that multiple complexes were involved in SUMO-dependent repression by Sp3 [64]. Among the genes identified whose knockdown impaired SUMO-dependent transcription repression were genes encoding the ATP-dependent chromatin remodeler dMi-2, the Drosophila ortholog of the nematode protein MEP-1 and the polycomb protein Sfmbt. Biochemical analyses indicated that dMi-2, MEP-1 and Sfmbt interacted with each other, bound to SUMO and were recruited to the chromatin in a SUMO-dependent manner. In addition, chromatin immunoprecipitation experiments showed that sumoylated Sp3 recruits a number of heterochromatin associated proteins, including dMi-2, the H3K9 histone methyl transferase (HMT) SETDB1, the H4K20 histone methyl transferase SUV4-20H, heterochromatin protein 1 (HP1) α, ß and γ, and MBT-domain proteins [141].

It has been previously indicated that dMi-2 is the core component of NuRD complex, a complex with associated HDACs. However, Sp3-SUMO-mediated repression is not senssistive to HDACs inhibitors, indicating either that dMi-2 mediates repression outside the NuRD complex or that there is a redundancy in the mechanisms driving Sp3-SUMO-mediated repression. In fact, several data indicate that dMi-2 is also part of another complex lacking HDAC activity. This complex, dMec, is composed by dMi-2 and the Drosophila homolog of the *C. elegans* protein MEP-1 (dMEP-1), and works as a corepressor of proneural genes [142]. Knockdown of dMEP-1 leads to derepression of Sp3 target genes, which is in contrast to functional redundancy among the different repression mechanisms recruited to Sp3 [64]. It is worth noting that MEP-1 was previously shown to contribute to SUMO-dependent repression in *C. elegans* [143]. Thus, sumoylated LIN-1 recruits MEP-1 for repression and inhibition of vulval cell fate. As LIN-1 is homologous to the human Elk-1, it is tempting to speculate that a similar mechanism may account for the SUMO-mediated HDAC-independent repression by Elk-1, despite the absence of a clear MEP-1 homolog in vertebrates.

As formerly mentioned, two HMTs were also recruited to SUMO-Sp3: SETDB1 and SUV4-20H, while the HMT SUV39H1 was not associated [141]. These HMTs were shown to be recruited to the *Dhfr* promoter in a sumoylatable Sp3-dependent manner. Knocking down of *SETDB1* and *SUV4-20H* resulted in reduced trimethylation of H3K9 and H4K20 at the *Dhfr* promoter.

Finally, polycomb protein Sfmbt and the corresponding mammalian orthologs L3MBTL1 and L3MBTL2 also associate to sumoylated Sp3 [64, 141]. These proteins contain repeats of the malignant brain tumor (MBT) domain, which is structurally related to the chromodomain and the Tudor domain, and like these, is able to recognize methylated histones. However, MBTs associate with higher affinity to mono- and di- than to trimethylated histones [144]. It has been shown that L3MBTL1 binds HP1γ and compacts chromatin in a mono- and dimethylated H4K20 and H1bK26-dependent manner [145]. Therefore, this association provides a way to explain L3MBTL1-mediated repression. Binding of HP1α, ß and γ to Sp3 depends on sumoylation [141]. Sumoylated histone H4 recruits HP1γ [55], and HP1α has also been shown to preferentially bind sumoylated SP100 [146], suggesting that, as occurs for HDACs, SUMO mediates HP1 recruitment. As Sfmbt, the PRC2-associated PcG protein Scm also contains MBT

repeats. In contrast to Scm, Sfmbt together with Pleiohomeotic, integrates in the polycomb complex PhoRC. Thus, different polycomb complexes include MBT-containing subunits, which might be involved in recognition of mono- and dimethylated histones to facilitate trimethylation by recruiting other subunits with histone methyltransferase activity.

In sum, Sp3 constitutes a paradigm of SUMO-dependent transcription repression through a variety of factors and chromatin-associated complexes. Clear evidence of SUMO involvement in Sp3-mediated repression came from the generation of knock-in mice with a non-sumoylatable version of Sp3 [147]. As Lys residues are targets for other modifications different of sumoylation, for instance acetylation, authors, instead of mutating core Lys551 to Arg changed the acidic residue at the sumoylation site. Interestingly, they substituted Glu by Asp, which abrogated sumoylation, despite for may authors it is assumed the consensus ΨKxE/D. Mutation did not affected Sp3 protein levels. However, spermatocyte-specific genes *Dmc1* and *Dnahc8*, and neuronal genes *Paqr6*, *Rims3* and *Robo3* appeared derepressed in non-testicular and extra-neural tissues and in mouse embryonic fibroblasts [147]. This correlated with loss of the repressive heterochromatin marks trimethyl-H3K9 and trimethyl-H4K20 and affected recruitment of repressor proteins, such as HP1, SETDB1, CHD3, and L3MBTL1/2, to the corresponding promoters. Surprisingly, homozygous knock-in mice born at expected mendelian frequency, were fertile and exhibited no obvious phenotype, in contrast to mice lacking Sp3 [148], suggesting that additional mechanisms may control protein expression from the aberrantly induced transcripts.

5. Conclusions

Sumoylation results essential for development and growth of all the investigated eukaryotes. In mice, embryos lacking the SUMO conjugating enzyme Ubc9 die at the early postimplantation stage, highlighting the relevance of SUMO conjugation during development [149]. The SUMO pathway is conserved from yeast to human and, together with ubiquitination, appears to be the most utilized pathway in post-translational modifications by UBLs. Despite similar structural features and a common evolutionary origin of SUMO and ubiquitin, they have significantly diverged from a functional point of view. In fact, a complete machinery has evolved around SUMO for specific conjugation/deconjugation of this molecule. Compared with ubiquitin, about 20 N-terminal extra amino acids are present in SUMO, which should account for the different and specific SUMO roles. From the many examples of protein modification by SUMO, structural, regulatory, signaling, and scaffold roles are inferred for this molecule. All these aspects convene to reveal SUMO modification as an important post-translational modification involved in transcription repression. Therefore, SUMO prefigures as an adaptor molecule essential for correct assembly and function of a variety of chromatin-associated repressor complexes. This does not exclude that involvement of SUMO in various systems results in transcriptional activation. A number of SUMO-dependent histone modifications and chromatin remodeling activities have been summarized in this chapter (Table 3). They include, HDACs, HMTs and histone demethylase activities, associated to the NuRD, LSD1/CoREST, SETDB1, dMec, L3MBTL1 and Polycomb complexes, which result in chromatin

compaction and gene silencing. However, many questions remain open. For instance, whether proteins with intrinsic repression activity like HDACs are directly recruited by SUMO or instead, relevant repression activity in vivo results from association of HDACs to repressor complexes recruited in a SUMO-dependent manner, needs to be clarified. In addition, although HDACs have intrinsic repression activity, it has been shown that sumoylation of HDAC1 accounts for its full repression activity [122], raising the question whether SUMO modulates its activity or is recruiting additional repressors. Another intriguing aspect concerns functional redundancy among the different repressors recruited to a locus via SUMO. A number of repressors are recruited to the chromatin through a Gal4-SUMO2 fusion [123], but it has been shown that individually knocking down of these factors has little consequences in SUMO2 displayed repression, which may be explained by functional redundancy of the multiple repressors associated. In a similar way, downregulation of CHD3 (mammalian dMi-2) or L3MBTL1/2 does not impair Sp3-SUMO-mediated repression in vertebrate cells [64, 141]. However, mutation of *dMi-2* or *Sfmbt* in Drosophila has a significant impact in Sp3-SUMO-dependent repression [64], suggesting that promoter context and local features account for the level of functional redundancy of SUMO-associated repressors. In addition, an important aspect of the SUMO modification concerns the fleeting nature of the modification in many cases, which means that SUMO-SIM interactions may have permanent consequences despite they are not further detected, a notion that implies a kind of memory and that thereby links SUMO to epigenetics. Interestingly, mutation of the SUMO2 SIM in CoREST is sufficient to abrogate repression of some neuronal specific genes in non-neuronal cells [111], highlighting the relevance of the non-covalent interaction of proteins with SUMO in regulating SUMO-dependent repression. In this context, SIMs and sumoylation sites have been described in many subunits within a repressor complex (reviewed in [68]), which rises the question about how the appropriate connections are established.

Abbreviations

CBP, CREB binding protein

DeSI-1, desumoylating isopepyidase-1

EZH2, Enhancer of Zeste

HDAC, histone deacetylase

HMT, histone methyl transferase

KAP-1, KRAB associated protein-1

NDSM, negatively charged residues-dependent SUMO motif

Pc2, polycomb-2

PcG, polycomb group

PCR1/2, polycomb repressive complex 1/2

PDSM, phosphorylation-dependent SUMO motif

PIAS, protein inhibitor of activated STAT

PML, promyelocytic leukaemia protein

SENP, sentrin-specific protease

SIM, SUMO interacting motif

Sp3, specificity protein 3

SP-RING, Siz/PIAS-RING

SUMO, small ubiquitin-like modifier

UBLs, ubiquitin-like proteins

Acknowledgements

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