SUMO and Chromatin Remodeling

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Abstract

Many of the known SUMO substrates are nuclear proteins, which regulate gene expression and chromatin dynamics. Sumoylation, in general, appears to correlate with decreased transcriptional activity, and in many cases modulation of the chromatin template is implicated. Sumoylation of the core histones is associated with transcriptional silencing, and transcription factor sumoylation can decrease gene expression by promoting recruitment of chromatin modifying enzymes. Additionally, sumoylation of transcriptional corepressors and chromatin remodeling enzymes can influence interactions with other transcriptional regulators, and alter their enzymatic activity. In some cases, proteins that are components of transcriptional corepressor complexes have been shown to be SUMO E3 ligases, further emphasizing the integration of sumoylation with the regulation of chromatin remodeling. Despite the evidence suggesting that sumoylation is primarily repressive for access to chromatin, recent analyses suggest that protein sumoylation on the chromatin template may play important roles at highly expressed genes. Elucidating the dynamic interplay of sumoylation with other post-translational modifications of histones and chromatin associated proteins will be key to fully understanding the regulation of access to the chromatin template.

Keywords

SUMO • Chromatin • HDAC • Polycomb • Transcription

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Abbreviations

SUMO	small ubiquitin like modifier
Ubc9	ubiquitin-conjugating enzyme 9
HAT	histone acetyl transferase
HDAC	histone deacetylase
HP1	heterochromatin protein 1
MAR	matrix attachment region
PML	promyelocytic leukemia protein
PIAS	protein inhibitor of activated STAT
RING	really interesting new gene (a zinc binding domain)
SP-RING	Siz/PIAS RING
PRC	polycomb repressive complex
CBX	chromobox

3.1 Introduction

A large number of proteins involved in the regulation of transcription and chromatin accessibility are substrates for modification by SUMO. Numerous transcription factors themselves have been shown to be sumoylated, and in general, this results in decreased transcriptional activation (Ouyang and Gill 2009). The nucleosome, which forms the basic repeating unit of chromatin, consists of DNA wrapped around a histone octamer (Luger and Hansen 2005). Arrays of regularly spaced nucleosomes are packaged into chromatin fibers, which include other histone binding proteins, as well as linker histones. Within the eukaryotic nucleus, chromatin is further organized into higher order structures. Transcriptionally silent heterochromatin is often localized to the nuclear periphery, and is interspersed with nuclear domains enriched for active chromatin (Akhtar and Gasser 2007). The complex patterns of histone modifications, such as acetylation, methylation and phosphorylation, have led to the histone code hypothesis (Jenuwein and Allis 2001; Strahl and Allis 2000). Histones act as platforms to which modifications are added, and the combinations of modifications are then read by protein complexes which bind to specifically modified histones (Ruthenburg et al. 2007). In addition to the more extensively studied histone modifications, such as lysine acetylation and methylation, histones can also be ubiquitinated on specific lysine residues, further expanding the complexity of this signaling platform (Robzyk et al. 2000; Zhang 2003). It is also clear that the histones are targeted for sumoylation, and that this can have direct effects on DNA accessibility and gene expression (Nathan et al. 2006; Shiio and Eisenman 2003). Transcription factors, and many other chromatin-associated proteins are also known to be sumoylated, expanding the role of SUMO in governing access to the chromatin template. Genome-wide analysis of SUMO distribution suggests that rather than being simply a repressive mark, SUMO modification of the chromatin template and associated proteins may play a more complex and dynamic role in regulating expression of highly transcribed genes (Liu et al. 2012; Neyret-Kahn et al. 2013; Niskanen et al. 2015; Seifert et al. 2015). Here we discuss the links between the sumoylation machinery and chromatin remodeling, primarily with respect to the regulation of transcription.

3.2 Histone Sumoylation

Direct modification of the histones themselves by SUMO is the simplest model by which sumoylation can modulate chromatin dynamics. Histone sumoylation was first demonstrated in mammalian cells, where sumoylation was detected predominantly on histone H4, but is also found to some degree on all four core histones (Shiio and Eisenman 2003). Sumoylation of H4 increased its interaction with a histone deacetylase (HDAC1) and with HP1γ (heterochromatin protein 1), suggesting a repressive role for histone sumoylation. Somewhat surprisingly, SUMO was detected on acetylated histone H4, and over-expression of the p300 transcriptional coactivator, which has histone acetyl transferase (HAT) activity, increased H4 sumoylation. In

these analyses, acetylation was detected with an antibody which recognizes acetylated lysines 5, 8, 12 and 16 in H4, and it was not known which lysines were sumoylated (Shiio and Eisenman 2003). It is, therefore, possible that sumoylation of H4 at another lysine might override the effects of acetylation at one or more of these lysines. However, despite the apparent contradiction between the activating (acetyl) and repressing (SUMO) modifications, this clearly suggests that other histone modifications can influence H4 sumoylation.

Further evidence for the direct regulation of chromatin dynamics via direct histone sumoylation comes from the budding yeast, *S. cerevisiae* (Nathan et al. 2006). All four yeast core histones are sumoylated, and sumoylation is associated with transcriptional repression. In contrast to the specific modifications that are associated with transcriptional repression in mammalian cells, the major characteristic of transcriptional silencing in budding yeast is histone H3 and H4 hypoacetylation; a lack of modification, rather than the presence of repressive modifications (Berger 2002). Thus, the identification of histone sumoylation as a specific modification, which promotes silencing in *S. cerevisiae* helped fill this apparent gap. Histone sumoylation is enriched at telomeres (Nathan et al. 2006), which are maintained in a transcriptionally silent state, associated with low levels of histone acetylation and ubiquitylation (Rusche et al. 2003). Histone sumoylation was also found at inducible genes in the uninduced state, and the level of histone sumoylation was shown to decrease with transcriptional induction. A reciprocal pattern of H2B sumoylation and acetylation of lysine 16 is seen at inducible genes, again implying a dynamic interplay between sumoylation and other histone modifications. For the sumoylation of mammalian histones, no E3 has been identified. In contrast, in *S. cerevisiae*, histone sumoylation is enhanced by the Siz1 and Siz2 ligases, which are the major E3s in this yeast (Johnson and Gupta 2001; Nathan et al. 2006).

For *S. cerevisiae* H4 and H2B, it appears that one or more of several lysines can be modified. In H4, lysines 5, 8, 12, 16 and 20 are the major SUMO acceptors, and in H2B, two pairs of lysines (either K6/7 or K16/17) within the repeated AEKKPA motif are modified (Nathan et al. 2006). SUMO acceptor lysines have been identified in mammalian histones H3 and H4 via large-scale proteomic approaches (Galisson et al. 2011), but there has not been an extensive analysis of which lysines are modified. In one analysis, histone H4 K12 and H3.1 K24 were found to be modified by SUMO. In addition to the core histones, the linker histone, H1, and the variant histones, H2AX and H2A.Z have been shown to be sumoylated, with the latter two playing a role in the DNA damage response (Chen et al. 2013; Galisson et al. 2011; Kalocsay et al. 2009; Matafora et al. 2009; Shiio and Eisenman 2003). The SUMO acceptor lysines which have been identified in the histones do not conform to the classical SUMO consensus site (KxE; (Melchior 2000)). Interestingly, the best match to this site (lysine 79 in histone H3 [FKTD]), is conserved from yeast to mammals, and has been shown to be a site of modification by the Dot1 family of methyltransferases, so is clearly accessible for modification (Ng et al. 2002; van Leeuwen et al. 2002). As with the majority of other known histone modifications, sumoylation occurs primarily within the flexible amino-terminal tails, favoring a model in which sumoylation and other modifications, such as acetylation, may compete either for individual lysines, or specific histone tails. Alternatively, histone sumoylation might result in the recruitment of other histone modifying proteins, such as HDACs, to further modify the chromatin template. Recent work examining chromatin compaction with nucleosomes that were homogenously modified by SUMO3 at H4K12 suggests an additional level of regulation (Dhall et al. 2014). In this analysis the addition of SUMO3 inhibited the higher order compaction of nucleosome arrays by preventing internucleosome interactions. In this model, the addition of SUMO might then be expected to favor chromatin accessibility by preventing compaction of the chromatin template. However, it should be noted that this work was carried out

with uniformly modified nucleosomes, and it is likely that *in vivo* modifications would be more sporadic. While it is clear that histones are modified by SUMO, the outcomes are less well understood, and it is possible that histone sumoylation plays different roles at different loci or in different physiological settings. The potential interplay of histone sumoylation with other histone modifications suggests that sumoylation contributes to the dynamic mechanism by which combinatorial histone modifications modulate access to the DNA template.

3.3 SUMO and Higher Order Chromatin Structure

While histone sumoylation may affect chromatin packaging at the most basic level, changes in nuclear organization may also alter chromatin structure and accessibility. The sumoylation machinery is important for overall nuclear integrity (Heun 2007), and for the formation of subnuclear structures, such as PML bodies (Muller et al. 1998; Shen et al. 2006). Evidence for a role for SUMO in higher order nuclear structure comes from targeted mutation of the mouse *Ubc9* gene. This mutant results in embryo inviability soon after implantation, and defects in chromosome condensation and segregation in mutant blastocysts cultured *in vitro* (Nacerddine et al. 2005). Additional defects in nuclear structure, including disruption of PML bodies, nucleoli, and the nuclear lamina were also observed in the absence of Ubc9. While some of these effects are consistent with SUMO playing a major role in the regulation of chromatin structure and overall nuclear architecture, it is also possible that the causes could be more indirect. For example, defects in nuclear transport in the absence of Ubc9 might have profound effects on nuclear architecture by altering the import of proteins required to maintain nuclear integrity (Melchior et al. 2003).

In mammalian cells, SUMO and sumoylated proteins have been observed to colocalize with highly heterochromatic regions in the nucleus. During meiosis in male cells, the sex chromosomes are packaged into the XY body, a specialized chromatin domain that is transcriptionally silent and does not undergo recombination. Although the precise function of the XY body is not clear, it may be involved in maintaining gene silencing and preventing potentially deleterious recombination events between the sex chromosomes (Handel 2004). In addition to colocalization of SUMO itself with the XY body, sumoylated proteins including Daxx and PML associate with this specialized chromatin domain (Rogers et al. 2004). SUMO can also be found localized to constitutive heterochromatin, and specifically to the regions of centromeric heterochromatin on human chromosomes 1 and 9, during meiosis (Brown et al. 2008; Metzler-Guillemain et al. 2008).

In *Drosophila* SUMO can be seen both at discrete locations in euchromatic regions of the polytene chromosomes and at the chromocenter (Lehembre et al. 2000). The suppressor of position effect variegation, Su(var)2–10, encodes a *Drosophila* member of the PIAS family of proteins, which are SUMO E3 ligases (Hari et al. 2001). Although PIAS proteins may have functions other than as E3s, this clearly raises the possibility that sumoylation regulates chromatin structure in flies. Additionally, a role has been demonstrated for sumoylation in the regulation of the gypsy insulator in flies (Capelson and Corces 2006). Insulators are thought to act as chromatin organizers, which establish distinct chromosomal domains, such that gene expression can be independently regulated in adjacent domains (Bushey et al. 2008). Two components of the *Drosophila* gypsy insulator can be sumoylated, and on polytene chromosomes SUMO associates with a fraction of the insulators (Capelson and Corces 2006). Interestingly, mutations in the fly genes encoding the SUMO E2 or SUMO itself suppressed the effects of mutations in components of the gypsy insulator. This suggests an antagonistic role for SUMO in insulator function, which may be explained by decreased clustering of insulators when sumoylated. There is also evidence that mamma-

lian CTCF, which has insulator function, is sumoylated dependent on the Pc2/CBX4 E3, suggesting that sumoylation may affect insulator function in mammals (Macpherson et al. 2009).

Matrix attachment regions (MARs) and MAR-binding proteins play a role in integrating global chromatin organization with the regulation of gene expression (Bode et al. 2000). In pre-B cells the MARs of the immunoglobulin μ locus are bound by the special AT-rich sequence binding protein 2 (SATB2), resulting in increased gene expression (Dobreva et al. 2003). SATB2 sumoylation, which is promoted by PIAS1, affects both the sub-nuclear localization of SATB2 and its ability to regulate gene expression. Mutation of the SUMO acceptor lysines within SATB2 decreased its association with the nuclear periphery, a localization that could be restored by fusion to SUMO3 (Dobreva et al. 2003). The T cell specific SATB1 has also been shown to be sumoylated, although in this case sumoylation increased the caspase mediated cleavage of SATB1 during apoptosis, suggesting that multiple regulatory mechanisms may be controlled by SUMO (Tan et al. 2008). Additional MAR associated proteins, including SAFB (scaffold attachment factor B) and SAFB2 have been shown to be sumoylated, and the PIAS1 E3 can promote modification of SAFB1 (Garee et al. 2011; Liu et al. 2015). There is evidence for both positive and negative effects of SAFB sumoylation on gene expression, perhaps consistent with the MAR-binding proteins functioning to modify the effects of other transcriptional regulators.

The effects of SATB2 sumoylation on gene expression and localization may be linked since inactive genes often preferentially localize to the nuclear periphery (Akhtar and Gasser 2007). For example, in *S. cerevisiae* the Siz2 SUMO E3 regulates the perinuclear tethering of telomeres, and this is likely dependent on sumoylation of components of the Sir complex or of Yku70/80 (Ferreira et al. 2011). Thus it appears that sumoylation may regulate the function of insulators and MARs, and likely plays an important role in regulating the partitioning of chromatin domains and of their positioning within the nucleus.

3.4 Telomeres and Centromeres

Centromeres and telomeres are specialized chromatin domains with roles in chromosome structure and maintenance, which have also been extensively studied for effects of chromatin structure on transcriptional regulation. Sumoylated histones are enriched at the telomeres in *S. cerevisiae*, correlating with transcriptional repression at these loci (Nathan et al. 2006). In addition, there is evidence from the fission yeast, *S. pombe*, and from *S. cerevisiae* that SUMO plays a role in maintaining chromatin structure at both centromeres and telomeres. Indeed, the essential *S. cerevisiae* gene, *SMT3*, which encodes the single yeast SUMO was first identified as a high copy suppressor of mutations in the *MIF2* gene, which encodes a centromere binding protein (Meluh and Koshland 1995). In addition to Siz1 and Siz2, Zip3 and Mms21 are also SUMO E3 ligases in *S. cerevisiae* (Cheng et al. 2006; Zhao and Blobel 2005). Zip3 plays a role in the formation of the synaptonemal complex during meiosis. Mms21 was found to copurify with a DNA repair complex, which included the Smc5 and Smc6 (structural maintenance of chromosomes) proteins (Zhao and Blobel 2005). Smc5 and the yeast DNA repair protein, Yku70, were both shown to be sumoylated substrates of Mms21. Mutation of *MMS21* resulted in a number of nuclear phenotypes, including increased DNA damage sensitivity and defects in telomeric silencing and length regulation. Human MMS21 is also a SUMO ligase, which promotes sumoylation of DNA repair proteins including SMC6 and TRAX, and is required for efficient DNA repair (Potts and Yu 2005). In addition to effects on telomeric silencing, there is evidence that sumoylation can regulate telomere length in yeast. SUMO modification of a component of the shelterin complex in *S. Pombe* prevents accumulation of telomerase and maintains normal telomere length regulation (Miyagawa et al. 2014), and sumoylation of Cdc13 is required for telomere length regulation *S. cerevisiae* (Hang et al. 2011). In *S. pombe*, there are two known SP-RING family SUMO E3s, Pli1 and Nse2 (Watts et al. 2007). Deletion of Pli1 does not result in a severe growth defect

(unlike mutations in the genes encoding the *S. pombe* SUMO and Ubc9 homologs), but causes decreases in global sumoylation (Xhemalce et al. 2004). Cells lacking Pli1 showed alterations in both telomere and centromere homeostasis. Telomeres elongated via what appeared to be a Rad51-dependent gene conversion-like mechanism, minichromosome instability was increased, and reporter genes integrated at centromeres were lost by gene conversion (Xhemalce et al. 2004). Additionally, telomerase activity can be increased by inhibition of the SUMO pathway, dependent on Pli1, but not Nse2 (Xhemalce et al. 2007). In cells lacking Pli1, there was also a decrease in transcriptional silencing at centromeres, further pointing to changes in chromatin structure (Xhemalce et al. 2004). As with yeast, a link to the regulation of telomere homeostasis has been uncovered in mammalian cells. In certain cancer cells, which obtain abnormally long telomeres by recombination rather than increased telomerase activity, telomeres have been found clustered at PML bodies (Yeager et al. 1999). In these cells, the SMC5/6-MMS21 complex was found to colocalize with the PML-telomere clusters, and the telomere binding proteins RAP1 and TRF2 were shown to be sumoylated in a MMS21-dependent manner (Potts and Yu 2007). Mutations in TRF2, which abolished its sumoylation led to decreased localization at PML, and experimentally decreasing expression of MMS21 resulted in shorter telomeres. Based on studies from yeast to humans, it appears that sumoylation may play multiple roles at telomeres, directly regulating chromatin structure at the level of the histones themselves, and also regulating higher order telomere structure, via the modification of telomere binding proteins and proteins involved in length regulation and end protection.

In *S. pombe*, centromeric heterochromatin has been extensively studied, and many of the components involved in its maintenance have been identified (Grewal and Jia 2007). Swi6, and the paralogous Chp2, are members of the HP1 family (Lomberk et al. 2006), which bind to methylated lysine residues on histone H3, via their conserved chromodomains (Jacobs and Khorasanizadeh 2002; Nielsen et al. 2002; Bannister et al. 2001). Clr4, which is a relative of the *Drosophila* Su(var)3–9 suppressor of position effect variegation, is a lysine methyl transferase. Clr4 methylates lysine 9 of histone H3, facilitating binding of HP1-like proteins, such as Swi6, and the spreading of heterochromatin (Rea et al. 2000). Mutations in the *pmt3* gene, which encodes the *S. pombe* SUMO, result in decreased silencing of a reporter gene inserted either at the silent *mat3* mating type locus, or at the centromere (Shin et al. 2005). This decreased silencing is associated with an increase in levels of histone H3 methylated at lysine 4, a mark of actively transcribing chromatin. A model for the role of SUMO at heterochromatic regions in *S. pombe* has been suggested, in which it is sumoylation of histone binding proteins, which regulates the association of these proteins with chromatin (Shin et al. 2005). The Ubc9 homolog, encoded by *hus5*, associates with heterochromatic regions, dependent on Clr4 and Swi6. Both Clr4 and Swi6 interact with Hus5 and both proteins can be sumoylated. Importantly, non-sumoylated mutants of Swi6 showed reduced silencing, with some decrease in the amount of Swi6 recruited to the silent loci. Thus it appears that recruitment of the SUMO E2 to silent regions, via interactions with proteins such as Clr4 and Swi6, may allow for their sumoylation, which in turn contributes to the stable maintenance of heterochromatin structure. In this context, it is of interest that in mammalian cells, targeting Ubc9 to DNA via a heterologous DNA binding domain resulted in an increase in the amount of both SUMO and HP1γ at chromatin, together with reduced histone H3 acetylation on lysines 9 and 14 (a mark of actively transcribing genes) (Shiio and Eisenman 2003). Unlike at the *S. pombe* centromeric heterochromatin, in this case it may be that direct sumoylation of the histones was the outcome. In mammalian cells, there is also evidence for recruitment of HP1 proteins to pericentric heterochromatin, dependent on the SUMO pathway. At centromeric and pericentric chromatin, HP1 pro-

teins bind to trimethylated H3K9, which is a mark of repressed chromatin. *De novo* targeting of HP1α was found to require its modification by SUMO1, and this recruitment of modified HP1 was via interactions with a long non-coding RNA derived from the pericentric alpha-satellite DNA (Maison et al. 2011). This requirement for HP1 sumoylation appears to be transient, with SUMO modification being dispensable for maintenance of HP1 at pericentric heterochromatin. Indeed, depletion of a SUMO protease (SENP7) that was able to de-sumoylate HP1α resulted in loss of HP1α from these chromatin domains, suggesting a requirement for transient rather than stable modification (Maison et al. 2011, 2012). However, it is also possible that SENP7 plays a more structural role in maintaining HP1 at heterochromatin, by bridging interactions between adjacent HP1 molecules, with the de-sumoylation activity of SENP7 being less important (Romeo et al. 2015).

There is considerable evidence that sumoylation plays roles in regulating chromatin structure and in the assembly or maintenance of specific chromatin domains. However, the precise functions of sumoylation are not always clear and it appears that, depending on the protein that is modified and the loci under consideration, sumoylation can have what appear to be opposing effects on access to the chromatin template. Such apparently contradictory effects may also reflect differential and transient requirements for sumoylation at different steps of a process.

3.5 SUMO-Dependent Recruitment of General Transcriptional Corepressors

Sumoylation provides an attractive model for modulating protein recruitment, particularly with the identification of non-covalent SUMO-interaction motifs (SIMs), which may facilitate protein interactions dependent on the sumoylation of one partner (Hannich et al. 2005; Minty et al. 2000; Song et al. 2004, 2005). These primarily hydrophobic patches in SUMO-interacting proteins interact with relatively low affinity with SUMO, but when present in multiple copies, or together with other interaction domains, can contribute significantly to protein interactions. Thus sumoylated proteins that are associated with the chromatin template may function as recruitment signals for additional chromatin regulatory proteins.

One clear example of SUMO-dependent recruitment of a chromatin modifying activity operates for the ETS-related transcription factor Elk-1 (Yang and Sharrocks 2004). Sumoylation, primarily of a single site, within the transcriptional repression domain of Elk-1 is required for repression (Yang et al. 2003). Sumoylated Elk-1 is associated *in vivo* with histone deacetylase activity, and recruits HDAC2 via its sumoylated repression domain (Yang and Sharrocks 2004). Interestingly, in the case of Elk-1 the sumoylated repressive complex is thought to be poised at promoters, such that in response to mitogenic signals via the MAP kinase pathway, Elk-1 is desumoylated allowing for rapid activation of gene expression (Yang et al. 2003). This relatively simple model provides an important paradigm for SUMO-dependent repression, in which sumoylation of a transcription factor results in recruitment of a chromatin remodeling activity to specifically repress target gene expression. Additionally, the regulated removal of SUMO allows for the reversibility of this switch, from repression to activation.

A similar model for the inhibition of transcriptional activation by the transcriptional coactivator, p300, has been proposed (Girdwood et al. 2003). In this case it is the class II histone deacetylase, HDAC6, which is recruited in a SUMO-dependent manner resulting in inhibition of transcriptional activation. HDAC6 is recruited to a region in p300, which acts as an independent transcriptional repression domain, and is separate from the HAT domain required for transcriptional activation. Regulation of HDAC recruitment by sumoylation has been identified for an increasing number of transcriptional regulators, including the p68 DEAD box RNA helicase, and Reptin, which is a component of a chromatin remodeling complex with links to tumor progression. The

sumoylated form of p68 represses gene expression likely via the preferential recruitment of HDAC1 (Jacobs et al. 2007). Similarly, Reptin interacts with HDAC1 in a sumoylation dependent manner (Kim et al. 2006). Thus, both transcription factors and transcriptional coregulators can be modified by SUMO to convert them to a more repressive form, via the recruitment of HDACs. However, particularly with histone deacetylases, the possibility exists that it is not just the chromatin template that is being targeted for deacetylation, but the regulatory proteins themselves. HATs have long been known to acetylate other proteins in addition to histones, and in some cases this is known to be reversed by specific HDACs (Glozak et al. 2005).

There is evidence that other histone modifying activities and larger corepressor complexes can be recruited in a SUMO-dependent manner. Sumoylation of Sp3 at a single lysine residue blocks its ability to activate transcription (Ross et al. 2002; Sapetschnig et al. 2002), and this may in part be explained by the SUMO-dependent recruitment of a transcriptional repression complex (Stielow et al. 2008a). At least in some cell types, sumoylation of Sp3 also results in a redistribution of Sp3 to the nuclear periphery and nuclear foci, and it is possible that these two mechanisms may act in concert (Ross et al. 2002). However, mutation of the sumoylated lysine in Sp3 relieves repression, allowing Sp3 to activate transcription. Multiple proteins required for the SUMO-dependent inhibition of Sp3 transcriptional activation were identified in cultured *Drosophila* cells, including components of the sumoylation machinery and proteins with links to transcriptional repression (Stielow et al. 2008a). These included the ATP-dependent chromatin remodeling protein, Mi-2 and the related Chd3. In addition to their ATPase and helicase domains, both proteins also have PHD (plant homeodomain) and chromodomains. Mi-2 is a component of the NuRD (nucleosome remodeling and deacetylase) complex, a general transcriptional repression complex with both histone deacetylase and ATP-dependent nucleosome remodeling activities (Zhang et al. 1999). Mi-2 binds better to sumoylated Sp3 than to unmodified Sp3, and Mi-2 recruitment to chromatin was decreased in the presence of SUMO-mutant Sp3 (Stielow et al. 2008a). Also identified in this screen were MEP-1, a zinc finger containing protein, which in *C. elegans* is associated with Mi-2, and Sfmbt, which binds to methylated histones H3 and H4 (Klymenko et al. 2006; Unhavaithaya et al. 2002). Both were shown to interact with Mi-2 and bind sumoylated Sp3. This led to the model that these proteins form a transcriptional repression complex, which is targeted to sumoylated transcription factors. There is also evidence for a similar SUMO-dependent repression of Sp3 via Mi-2 recruitment in mouse cells, pointing to a conserved mechanism (Stielow et al. 2008a). Recruitment of this SUMO-dependent transcriptional corepressor complex results in local transcriptional repression by the formation of a heterochromatin like state, with increases in methylation of histone H3 on lysine 9, and H4 on lysine 20 (Stielow et al. 2008b). The *in vivo* importance of Sp3 sumoylation was demonstrated by the generation of a mouse line with a single amino-acid change in Sp3 that prevented sumoylation of the Sp3 transcriptional inhibitory domain (Stielow et al. 2010). This resulted in derepression of Sp3 target genes, together with reduced recruitment of corepressors, including Mi-2, and a reduction in repressive chromatin marks at the promoters of derepressed genes. The recruitment of large corepressor complexes to chromatin can be mediated in part by interaction of a SIM-containing component of the complex with a sumoylated transcription factor. For example, a CoREST1 complex that includes LSD1 and HDACs can interact with SUMO2 via a slightly divergent hydrophobic SIM in CoREST1. This results in recruitment of the CoREST1/LSD1/HDAC complex to sumoylated transcription factors and subsequent repression of transcription (Ouyang et al. 2009). Thus transcriptional silencing can be initiated by the sumoylation of a transcription factor, which in turn recruits the machinery to modify the chromatin template, thereby altering accessibility to other factors.

3.6 SUMO-Dependent Modulation of General Coregulator Activity

The recruitment of chromatin modifying complexes via a sumoylated transcriptional regulator is clearly a major way in which SUMO contributes to transcriptional regulation, as evidenced by the simple fact that artificially fusing SUMO to a transcription factor generally results in transcriptional repression (for example see (Ross et al. 2002)). However, other mechanisms have been proposed, such as the sequestration or relocalization of sumoylated proteins, as discussed for Sp3. Additionally, the transcriptional regulators CBP and Daxx have been shown to localize to PML oncogenic domains (PODs) in a SUMO dependent manner (Best et al. 2002). This localization prevents them from regulating transcription, however, once desumoylated by a SUMO protease, CBP and Daxx can be released from PODs allowing them to perform their gene regulatory functions.

The mechanisms discussed so far result in the inhibition of transcriptional activation by sumoylation, either by driving the recruitment of general repressors, or by removing the activator. However, sumoylation of general transcriptional corepressors, including HDACs, may alter their activity. Human HDAC1 can be sumoylated at two carboxyl-terminal lysines (David et al. 2002). Blocking sumoylation of HDAC1 resulted in decreased transcriptional repression, presumably by reducing histone deacetylation. Additionally, there is evidence that sumoylation affects HDAC1 stability, and this appears to be dependent on whether HDAC1 is modified by SUMO1 or SUMO2, suggesting both positive and negative effects of sumoylation (Citro et al. 2013). HDAC4 is a class II HDAC, which has a large amino-terminal domain with a high degree of similarity to the MITR transcription factor, and is known to be present in both nuclear and cytosolic compartments (Grozinger et al. 1999; McKinsey et al. 2001). Sumoylation of HDAC4 occurs at a single lysine close to the amino-terminus of its HDAC domain (Kirsh et al. 2002). Blocking HDAC4 sumoylation results in decreased deacetylase activity, suggesting that sumoylation is important for modulating deacetylase-mediated repression of gene expression. Although it is not clear how sumoylation of HDACs regulates deacetylase activity, it is possible that it has subtle effects on sub-cellular localization or interaction with other proteins that may alter HDAC activity. Support for sumoylation of general corepressors as a targeting mechanism comes from the sumoylation of HP1α driving recruitment to pericentric heterochromatin, as discussed earlier (Maison et al. 2011). Additionally, the lysine demethylase, KDM5B, is sumoylated and this results in increased occupancy at target genes, resulting in the demethylation of trimethylated H3K4 and transcriptional repression (Bueno and Richard 2013).

There is evidence for a more complex interplay of sumoylation and acetylation, which is dependent on HDAC4. MEF2 transcription factors can be sumoylated on a single conserved lysine, and this is increased by HDAC4, suggesting a role for HDAC4 as a SUMO E3 (Gregoire and Yang 2005; Zhao et al. 2005). Sumoylation of MEF2 at this site decreases its ability to activate gene expression. Interestingly, MEF2 can also be acetylated by the coactivator, CBP, on the same lysine at which it is sumoylated. In contrast to sumoylation, acetylation increases MEF2 activity (Zhao et al. 2005). The switch between acetylation and sumoylation of MEF2 is controlled by the class III HDAC, SIRT1, together with HDAC4. Thus, SIRT1 deacetyates MEF2, followed by HDAC4-dependent sumoylation of MEF2, together decreasing its transcriptional activation potential (Zhao et al. 2005). A similar mechanism for deacetylation followed by sumoylation, mediated by SIRT1 and HDAC4 has been demonstrated for HIC1, suggesting that this may be a more general mechanism (Stankovic-Valentin et al. 2007). In this case, sumoylated HIC1 can recruit the NuRD complex via interaction with MTA1, and NuRD complex recruitment can be inhibited by acetylation of HIC1 (Van Rechem et al. 2010). Thus, in addition to SUMO regulating the ability of HDACs to modify chromatin structure via the deacetylation of histones, there appears to be a more intricate

and complex interplay of sumoylation with other protein modifying activities emerging.

3.7 The Role of SUMO E3 Ligases in Chromatin Remodelling

Although sumoylation can occur in the absence of an E3 ligase, their presence can increase specificity and the efficiency of the sumoylation reaction (Johnson 2004). The presence of a SUMO E3 ligase as an integral part of a complex with chromatin modifying activity represents an efficient and specific way for sumoylation to regulate chromatin remodeling. One of the first SUMO E3s to be identified in mammalian cells was PIASy, a member of the SP-RING family of E3s (Sachdev et al. 2001). PIASy was shown to promote sumoylation of the transcription factor, LEF1, sequestering it at nuclear bodies and decreasing its ability to activate gene expression. PIASy was found to be present at MARs, perhaps suggesting a role in modulating higher order chromatin structure, a notion which is also supported by the fact that mutations in the gene encoding a member of the PIAS family have effects on position effect variegation in *Drosophila* (Hari et al. 2001). Indeed PIAS family members in mammalian cells are well characterized as transcriptional coregulators for multiple transcription factors, and they may perform other functions in addition to driving sumoylation of their interacting partners (Rytinki et al. 2009). The polycomb protein, Pc2/CBX4, was shown to be a SUMO E3 for the transcriptional corepressor, CtBP (Kagey et al. 2003). Pc2 was first identified based on its homology to the *Xenopus* homolog of *Drosophila* Pc, and was shown to localize to sub-nuclear foci, or polycomb bodies (Satijn et al. 1997). Polycomb foci have been observed in numerous cell types, and presumably are centers of heterochromatic transcriptional silencing. Indeed, polycomb proteins localize to regions of pericentric heterochromatin on human chromosome 1 (Saurin et al. 1998). Although the domains of Pc2/CBX4 required for E3 activity are well conserved among vertebrates (Kagey et al. 2005; Wotton and Merrill 2007), there is relatively little similarity of these domains to the founding member of the family, *Drosophila* Pc, suggesting that Pc2/CBX4 E3 activity is a vertebrate specific function. Additional SUMO substrates for Pc2 have been identified, including the *de novo* methyl transferase, Dnmt3a, the kinase HIPK2 and the zinc finger proteins, SIP1 and CTCF (Li et al. 2007; Long et al. 2005; Macpherson et al. 2009; Roscic et al. 2006). In most cases Pc2/CBX4 SUMO substrates colocalize at polycomb foci, raising the possibility that colocalization with Pc2 may contribute to maintaining substrate sumoylation. Although Pc2 has *in vitro* E3 activity (Kagey et al. 2003), it remains possible that *in vivo*, it also functions in part by protecting sumoylated proteins from desumoylation. Although relatively little is known about whether sumoylation contributes to polycomb body formation, it is tempting to speculate that SUMO plays a role, as it does with PML domains (Muller et al. 1998; Shen et al. 2006). Recent work has begun to suggest that the different functions of Pc2/CBX4 may regulate separate processes. Analysis of a role for Pc2/CBX4 in the homeostasis of epidermal stem cells and the epithelial identify of keratinocytes provides evidence that the highly conserved chromodomain, which binds methylated H3K9 and H3K27 (Bernstein et al. 2006) is required to limit cellular senescence. In contrast, SUMO mediated functions, dependent on the SIMs limit differentiation, and inactivating either function alone was shown to have differential effects on gene expression programs (Luis et al. 2011; Mardaryev et al. 2016). However, there is also evidence that H3K27Me3 binding and SUMO dependent activities within Pc2/CBX4 may be coordinated. Pc2/CBX4 is known to be sumoylated, and may function as an E3 for its own modification (Merrill et al. 2010). Recruitment of the Pc2/CBX4-containing PRC1 polycomb complex to the promoters of the *Gata4* and *Gata6* genes was shown to require the chromodomain binding to H3K27Me3, but this was modulated by sumoylation of Pc2/CBX4 (Kang et al. 2010). Desumoylation of Pc2/CBX4 driven by SENP2 reduced recruitment to the *Gata4* and *Gata6* genes, suggesting that sumoylated Pc2/CBX4

was better able to bind heterochromatin via its chromodomain.

Further support for the role of SUMO E3s as integral components of chromatin remodeling complexes comes from the analysis of the KAP1 transcriptional corepressor (also known as TIF1β) (Ivanov et al. 2007). KAP1 contains a PHD domain, which has some sequence similarity to the class of RING finger domains found in SUMO E3s (Hochstrasser 2001). In addition, KAP1 has an adjacent bromodomain, which binds acetylated histones H3 and H4. KAP1 is sumoylated within its bromodomain, dependent on the adjacent PHD domain, suggesting that this domain acts as an intra-molecular SUMO E3 (Ivanov et al. 2007). Sumoylation of the KAP1 bromodomain results in increased interaction with SETDB1 and the CHD3-containing NuRD complex. A model emerges in which sumoylation of KAP1 results in histone deacetylation, via the HDAC components of the NuRD complex. This is followed by SETDB1-dependent histone methylation, which facilitates binding of HP1 via its chromodomain to the methylated histone tails. Thus KAP1 SUMO E3 activity initiates a series of protein modifications, which result in the recruitment and spreading of HP1 to generate a locally silenced chromatin domain. One point to note with respect to chromatin associated SUMO E3s, such as KAP1, is that they may have relatively few SUMO substrates. This is in contrast to some members of the SP-RING family of E3s and RanBP2/Nup358, which may play much more general roles in sumoylation. The regulation of chromatin accessibility is modulated by protein complexes, which possess multiple enzymatic activities, including sumoylation. This can result in the modification of both histones and non-histone proteins in a coordinate manner, to regulate the accessibility of the DNA template. In summary, it is clear that sumoylation has joined the longer known protein modifications as a key regulator of chromatin dynamics. However, while much of the initial evidence suggested that sumoylation was repressive, there is emerging evidence that things may be more complex than this.

3.8 Global Analysis of Chromatin Modification by SUMO

In much of what has been discussed so far, the addition of SUMO results in decreased chromatin accessibility and transcriptional silencing. While this is clearly a major role of nuclear SUMO (Gill 2005), other possibilities should be considered. In *S. cerevisiae*, SUMO was found at the promoters of actively transcribed genes, but not at repressed genes (Rosonina et al. 2010). Activation of inducible gene expression was associated with increased SUMO and Ubc9 at their promoters. Inactivation of Ubc9 resulted in less promoter-associated SUMO and increased transcription due to a delay in shutting off gene expression. While this analysis focused on selected genes, it clearly raises the possibility that sumoylation on chromatin may play complex regulatory roles that cannot simply be defined as a repressive mark. This likely comes in part from the fact that sumoylation occurs not only, or even primarily, on histones, but on a large array of other chromatin associated proteins as well. It was later shown that the Gcn4 transcription factor was sumoylated at the promoters of inducible genes and that this facilitated its removal from chromatin following RNA polymerase II recruitment (Rosonina et al. 2012). Interestingly, the removal of Gcn4 was enhanced by the presence of the corepressor Tup1, which is also subject to regulation by sumoylation, suggesting that the coordinated modification of both activators and repressors by SUMO may be required (Ng et al. 2015). Thus unraveling functional consequences of chromatin sumoylation across the genome may require a knowledge of which proteins are conjugated with SUMO at each locus.

Several recent studies have analyzed the genome-wide distribution of SUMO in mammalian cells, and begun to address the consequences of sumoylation (Liu et al. 2012; Neyret-Kahn et al. 2013; Niskanen et al. 2015; Seifert et al. 2015). These analyses reveal some common themes, and also highlight some additional questions. In contrast to what might be expected,

genome-wide analyses found SUMO to be primarily localized to regions of active open chromatin. SUMO-enriched regions were more associated with activating chromatin marks such as H3K4 methylation, rather than with repressive histone modifications. This is perhaps in line with the idea that histone H4 sumoylation has been proposed to reduce nucleosome packing (Dhall et al. 2014). However, analysis of SUMO distribution by ChIP-seq likely reflects a combination of histone sumoylation and SUMO modification of other chromatin bound factors. In one study, the authors identify SAFB1 as one potential factor that is sumoylated at the promoters of highly transcribed genes, and further suggest that sumoylated SAFB1 promotes RNA PolII recruitment (Liu et al. 2012, 2015). An alternative suggestion is that SUMO enrichment at active promoters is due to sumoylation of components of the pre-initiation complex (Neyret-Kahn et al. 2013). Analysis of the effects of cellular stress on SUMO distribution may help explain the perhaps surprising finding that SUMO is primarily present at active genes (Niskanen et al. 2015; Seifert et al. 2015). One proposed function for the accumulation of SUMO2 at the transcriptional start sites of active genes is that sumoylation correlates with polymerase pausing and thereby limits the transcriptional response to heat-shock (Niskanen et al. 2015). In line with this, SUMO was found to be primarily correlated with the promoters of histone genes as well as those involved in protein biogenesis, and at PolI and PolIII transcribed genes, and depletion of SUMO resulted primarily in up-regulation of expression (Neyret-Kahn et al. 2013). The suppressive role of sumoylation suggested by these two studies is in contrast to the apparently activating role of SAFB1 sumoylation (Liu et al. 2015). However, on depletion of SUMO there were both increases and decreases in gene expression, as would be expected when examining transcription on a genome-wide scale. One possible explanation for apparently opposing effects of sumoylation is suggested by a second analysis of the response to stress (Seifert et al. 2015). Here, the authors show that SUMO2 is induced at active nucleosome depleted regions of the genome in response to heat-shock, but does not simply correlate with increased or decreased gene expression. Rather, they suggest that sumoylation acts to maintain the integrity of large chromatin bound protein complexes under conditions of stress, and possibly to some degree under normal cellular conditions. In this scenario, inhibition of sumoylation might activate some genes and repress others, depending on whether the particular gene is under the control of a regulatory complex that requires sumoylation for its integrity.

In summary, these recent analyses suggest that the role of sumoylation on the chromatin template is perhaps more complex than originally thought. However, they clearly point to SUMO as a regulator of highly expressed genes, and suggest that SUMO is primarily at relatively open chromatin regions. Any interpretation of the effects of SUMO depletion on genome-wide chromatin accessibility must be tempered by the potentially opposing effects of this modification on its multiple substrates. In the future it will be of interest to begin to dissect how sumoylation of specific chromatin associated proteins or protein complexes affects accessibility and downstream function.

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