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SUMO-mediated regulation of nuclear functions and signaling processes

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Summary

Since the discovery of SUMO twenty years ago, SUMO conjugation has become a widely-recognized post-translational modification that targets a myriad of proteins in many processes. Great progress has been made in understanding the SUMO pathway enzymes, substrate sumoylation, and the interplay between sumoylation and other regulatory mechanisms in a variety of contexts. As these research directions continue to generate insights into SUMO-based regulation, several mechanisms by which sumoylation and desumoylation can orchestrate large biological effects are emerging. These include the ability to target multiple proteins within the same cellular structure or process, respond dynamically to external and internal stimuli, and modulate signaling pathways involving other post-translational modifications. Focusing on nuclear function and intracellular signaling, this review highlights a broad spectrum of historical data and recent advances with the aim of providing an overview of mechanisms underlying SUMO-mediated global effects to stimulate further inquiry into intriguing roles of SUMO.

Brief overview of sumoylation and desumoylation processes

SUMO proteins are a family of conserved eukaryotic protein modifiers of approximately 100 amino acids. SUMO conjugation to the lysine(s) of substrates is carried out by SUMO E1, E2, and E3 enzymes (Johnson, 2004). Organisms examined so far contain only a single SUMO E1 and E2 enzyme but multiple SUMO E3 enzymes. The SUMO E1 uses ATP hydrolysis to covalently link SUMO to its active site cysteine and subsequently transfer SUMO to the active site on the E2. With the help of a SUMO E3 (or ligase), the E2 further transfers SUMO onto substrates (Fig 1A). SUMO is often conjugated at the sumoylation consensus sequence, ψKxE/D (ψ: hydrophobic residues; x: any amino acid; K: sumoylation site), which is recognized by the E2, or its reverse sequence (Rodriguez et al., 2001; Sampson et al., 2001). SUMO E3s support productive configurations for SUMO transfer by simultaneously binding the SUMO-charged E2 and the substrate (Streich and Lima, 2016; Werner et al., 2012). The multiple SUMO E3s within a cell have both distinct and overlapping substrates (Pichler et al., 2017). While lower eukaryotes contain only one

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SUMO, higher eukaryotes possess at least three SUMO isoforms, namely SUMO1–3. These isoforms differ in several respects, such as in their SUMO E3 preferences or ability to form poly-SUMO chains by conjugation of one SUMO to another SUMO molecule via different lysine residues (Pichler et al., 2017). Cell line studies further suggest that while the majority of SUMO1 is conjugated to substrates, SUMO2/3 mostly becomes conjugated under stress conditions. These differences suggest that SUMO isoforms can have distinct functions and regulation.

Once conjugated to substrates, SUMO can exert a variety of effects. These include changing substrate interactions with DNA, RNA or other proteins, altering conformation or enzymatic activities, and modulating other modifications (Flotho and Melchior, 2013). Among these effects, the most frequently described has been interactions between SUMO and SUMO interaction motifs (SIMs). Canonical SIMs contain a core of hydrophobic residues preceded or followed by negatively charged amino acids; they contact a hydrophobic pocket on SUMO with neighboring basic residues (Hecker et al., 2006; Song et al., 2004). The SUMO:SIM association is generally weak, but can be enhanced by the binding of multiple SIMs to SUMO chains. Effects of sumoylation can be reversed when the modification is removed by SUMO-specific proteases, or desumoylases, which are also functionally important (Hickey et al., 2012) (Fig 1A).

SUMO affects nuclear structures and functions

In the early 2000s, examination of SUMO and sumoylation machinery mutants captured some striking nuclear structural defects, such as fragmented nucleoli, declustered telomeres, and heterochromatin breakdown (Hari et al., 2001; Nacerddine et al., 2005; Shin et al., 2005; Xhemalce et al., 2004; Zhao and Blobel, 2005). Meanwhile, SUMO enzymes and sumoylated proteins were found to be enriched at nuclear structures, such as PML and Polycomb bodies (Kagey et al., 2003; Muller et al., 1998; Sternsdorf et al., 1997). These early findings hinted at the possibility that SUMO may globally affect biological processes via modulation of nuclear structures. Indeed, insights into cellular membraneless structures suggest that SUMO’s ability to facilitate protein-protein interactions can contribute to their formation. In general, proteins capable of intra- or inter-molecular multivalent interactions can form large oligomers and phase separate from the surrounding solution (Banani et al., 2017; Hyman et al., 2014). These proteins can then use their modular interaction domains or intrinsically disordered regions to recruit additional macro-molecules, expanding liquid droplets. These droplets and membraneless structures can undergo fusion, fission, and rapid molecular exchange with the surrounding solution, yet high concentrations of macro-molecules within the structures may promote certain biological processes (Banani et al., 2017).

The role of SUMO in phase separation and PML body formation

PML bodies provide an example of how SUMO:SIM interactions could contribute to the phase separation-mediated formation of nuclear structures. PML bodies host more than 150 proteins with a wide range of functions, such as DNA repair, stress response, senescence, antiviral immunity, and tumor suppression (Sahin et al., 2014). These proteins appear to

carry out some of their roles within PML bodies, though a unifying model for PML body functions remain to be established (Lallemand-Breitenbach and de The, 2018). It was noted early on that PML proteins and many other PML body constituents are sumoylated and contain SIMs; importantly, mutations affecting their sumoylation or SIMs were shown to impair PML body formation or constituent recruitment (Sahin et al., 2014; Shen et al., 2006). These findings and additional data suggested a model for PML body formation, wherein sumoylation of self-associated PML proteins recruits SIM-containing partner proteins, and sumoylation of the latter leads to additional SUMO:SIM interactions and PML body expansion (Banani et al., 2017; Sahin et al., 2014; Wang et al., 2018). Modeling SUMO:SIM interactions in engineered proteins shows that they are sufficient for driving phase separation *in vitro,* providing strong support for this model (Banani et al., 2016).

The effects of SUMO on nucleolar and proteinaceous structures

The nucleolus is the site of ribosome assembly, RNA processing, and cell cycle regulation, amongst other functions. Its domains also exhibit liquid droplet-like behaviors, such as diffusion and fusion (Brangwynne et al., 2011; Feric et al., 2016). How SUMO affects these behaviors has not been directly interrogated, but sumoylation and desumoylation both influence nucleolar structure and function. On one hand, nucleoli contain a proportion of sumoylation enzymes and substrates, and some of these enzymes and sumoylation events promote nucleolar integrity and functions (Ayaydin and Dasso, 2004; Heun, 2007; Matafora et al., 2009; Srikumar et al., 2013; Takahashi et al., 2008; Westman et al., 2010; Zhao and Blobel, 2005). On the other hand, desumoylation enzymes, such as SENP3 and 5 that target SUMO2/3-conjugates, are also enriched in nucleoli and are required for nucleolar function (Di Bacco et al., 2006; Finkbeiner et al., 2011; Liang et al., 2017; Yun et al., 2008). These data may suggest that specific sumoylation events and balanced sumoylation levels are required for nucleolar biology. Nucleoli additionally contain abundant ribosomal DNA units and RNA species capable of macromolecular association. A productive line of future inquiry may be exploring the potential collaborations between these DNA/RNA molecules and sumo in sculpting nucleoli.

While SUMO helps to “build up” membraneless organelles, it can also “break down” inactive proteinaceous structures, some of which underlie neurodegenerative diseases. A few examples include the ability of sumoylation to prevent protein inclusions formed by the transcriptional corepressor subunit Cyc8, to solubilize DNA end resection protein Sae2, and to reduce aggregation of translation factor CPEB3 or transcription factor androgen receptor (Drisaldi et al., 2015; Mukherjee et al., 2009; Oeser et al., 2016; Sarangi et al., 2015). Future study on how SUMO contributes to the dynamic assembly and disassembly of other proteinaceous and membraneless structures will expand our understanding of the structural roles of SUMO.

SUMO modulates chromosome structures and functions

SUMO and SUMO pathway enzymes associate with chromatin and SUMO substrates are enriched among DNA-binding proteins (Chymkowitch et al., 2015; Flotho and Melchior, 2013; Hendriks and Vertegaal, 2016; Neyret-Kahn et al., 2013; Niskanen et al., 2015). These

observations corroborate with early genetic data that SUMO deficiency drastically changes chromosome integrity and segregation (Hari et al., 2001; Nacerddine et al., 2005; Shin et al., 2005; Tanaka et al., 1999; Xhemalce et al., 2004). Recent studies further elucidated how dynamic sumoylation regulates specific chromosome structures, such as centromeres, telomeres, heterochromatin, and broken regions, as summarized below (Fig 1B).

Sumoylation and desumoylation are critical for centromeric structures and functions

Centromeres contain specialized histones and silenced chromatin and support the assembly of kinetochores for microtubule attachment during mitosis. SUMO pathway enzymes are enriched at centromeres and kinetochores in multiple organisms, and sumoylation of numerous proteins concertedly regulate centromeric structure and function as reviewed recently (Cubenas-Potts and Matunis, 2013). One highly conserved SUMO substrate is Topoisomerase II (or Top2) (Clarke and Azuma, 2017). Sumoylation of Top2 C-terminal non-catalytic region leads to centromeric recruitment of Top2 itself to decatenate intertwined DNA before anaphase, as well as other mitotic factors (Claspin, Haspin and Aurora B kinases) to promote centromeric segregation (Azuma et al., 2003; Bachant et al., 2002; Dawlaty et al., 2008; Edgerton et al., 2016; Ryu et al., 2010; Ryu et al., 2015a). The SUMO pathway also affects centromeric histones and other mitotic regulators (Mukhopadhyay and Dasso, 2017). For example, sumoylated Orc2 recruits the KDN5A demethylase to centromeres to convert H3K4me3 into H3K4me2, thus permitting non-coding RNA production from the locus and subsequent heterochromatin maintenance (Huang et al., 2016). In another recent example, SUMO removal was shown to help extract the Aurora B kinase from chromatin and relocate it to the spindle midzone, which is an essential transition during mitosis (Pelisch et al., 2014). How Aurora B is extracted is unclear, but may involve the Cdc48 segregase and cofactors, which use ATP hydrolysis to remove sumoylated proteins such as the centromeric histone CENP-A from DNA (Franz et al., 2016; Merai et al., 2014).

The important SUMO-based regulatory events exemplified above could explain the drastic chromosome segregation defects arising from acute chemical inhibition of SUMO E1 or depletion of SUMO E2 and specific desumoylases (He et al., 2017; Mukhopadhyay and Dasso, 2017; Nacerddine et al., 2005; Pelisch et al., 2014). Strikingly, aneuploidy-prone SUMO pathway mutants may produce adaptive situations wherein gaining an extra chromosome partially resets cellular homeostasis, as seen in yeast cells lacking the Ulp2 desumoylase (Ryu et al., 2016). How exactly aneuploidy can benefit cells and be maintained in this situation remains to be understood. Another future question to consider is whether centromeric regions and/or kinetochores experience SUMO-facilitated phase separation given the abundant SUMO-mediated interactions at these sites.

The SUMO pathway contributes to heterochromatin formation and maintenance

The SUMO pathway also regulates other heterochromatic loci in addition to centromeric regions. In particular, sumoylation of the heterochromatin assembly factor HP1 promotes its association with RNA transcripts located at these regions to achieve initial HP1 targeting (Maison et al., 2011). Subsequent HP1 propagation along heterochromatin involves its binding to H3K9me3, catalyzed by Suv39h1 (Bannister et al., 2001; Lachner et al., 2001).

Enhancement of HP1 sumoylation by Suv39h1 further provides a positive feed-forward mechanism in heterochromatin establishment (Maison et al., 2016). Sumoylation of the fission yeast HP1 homolog has also been implicated in heterochromatin regulation (Shin et al., 2005). Interestingly, interaction between SENP7 desumoylase and HP1 is important for maintaining the latter at heterochromatin, although the underlying mechanism is unclear (Maison et al., 2012). As such, temporal control of the HP1 sumoylation cycle appears to be key for heterochromatic structures. As HP1 phase separation is suggested to promote heterochromatin formation, addressing how its sumoylation and desumoylation are linked to this phenomenon will shed light on the potential effects of SUMO-based phase separation in chromatin domain dynamics (Larson et al., 2017; Strom et al., 2017).

SUMO is required for chromosome movement in multiple contexts

When heterochromatin experiences DNA double strand breaks (DSBs), sumoylation enables the broken DNA ends to relocate outside this region in order to prevent illegitimate repair such as those between repetitive sequences (Ryu et al., 2015b; Torres-Rosell et al., 2007). Analogously, SUMO helps target eroded telomeres and persistent DSBs in other parts of the genome to the nuclear periphery (Churikov et al., 2016; Horigome et al., 2016) (Fig 1B). Such targeting involves the association between sumoylated DNA repair proteins and SUMO-targeted ubiquitin ligases (STUbLs, more below) via SUMO:SIM interactions (Seeber and Gasser, 2017). Mechanisms enabling directional DNA end movement just began to emerge and involve a collaboration between nuclear actin and myosin with the Smc5/6 SUMO E3 complex (Caridi et al., 2018).

Studies highlighted in this section suggest a fundamental role for SUMO in the formation and movement of chromosomal structures (Fig 1B). Such a role could effectively modulate multiple forms of DNA transaction within these structures. In addition, SUMO is well known to regulate individual DNA transaction, such as transcription and DNA repair, by directly targeting proteins involved in these processes; as these topics have been extensively reviewed, we refer readers to some of these articles for details (Rosonina et al., 2017; Sarangi and Zhao, 2015; Schwertman et al., 2016; Wei and Zhao, 2017).

Global changes in sumoylation levels

Sumoylation states are highly dynamic, enabling rapid responses to changing external and internal stimuli. Studies from multiple organisms have begun to delineate the various mechanisms that enable large-scale changes of sumoylation levels and how this leads to alteration in multiple processes at once to achieve coordinated regulation. This section assesses three mechanisms and their implications.

STUbLs and their antagonists regulate sumoylation levels

The STUbL enzymes provide a major means of changing global sumoylation levels. Initially demonstrated in yeasts, it is now well accepted that STUbLs use their SIM arrays to bind poly-SUMO chains on proteins and poly-ubiquitinate these proteins, which often leads to their proteasome-mediated degradation (Mullen and Brill, 2008; Prudden et al., 2007; Sun et al., 2007; Uzunova et al., 2007; Xie et al., 2007). STUbLs target both SUMO pathway

enzymes and distinct groups of substrates (Fig 2A). For example, the yeast STUbLs can target Siz SUMO E3s; similarly a human STUbL, RNF4, targets SUMO E2 and multiple PIAS E3s that are heavily sumoylated (Kumar et al., 2017; Nie and Boddy, 2015; Westerbeck et al., 2014). In addition, RNF4 and another STUbL, Arkadia, affects the PML proteins. In particular, STUbL-mediated degradation of the PML-RARA fusion proteins, the causative agent for acute promyelocytic leukemia, underlies the therapeutic effect of arsenic in this disease (Erker et al., 2013; Lallemand-Breitenbach et al., 2008; Poulsen et al., 2013; Tatham et al., 2008). In another context, PML-mediated sumoylation of misfolded proteins located inside PML bodies can facilitate their RNF4-mediated degradation to reduce the protein aggregates associated with neurodegenerative disease (Chu and Yang, 2011; Guo et al., 2014). These findings corroborate the observation that proteasomes can be targeted to PML bodies when their subunits are sumoylated (Lamoliatte et al., 2017), suggesting that multiple SUMO-based mechanisms act in concert to prevent undesired protein aggregation.

As sumoylation is essential, STUbL-mediated degradation of sumoylated proteins must be reined in by counter-regulatory mechanisms to sustain SUMO homeostasis (Fig 2A). Recent studies suggest several means of antagonizing STUbLs. For example, the Ataxin and USP11 deubiquitinases counteract the effects of RNF4 in maintaining PML and DNA repair protein sumoylation levels (Hendriks et al., 2015; Pfeiffer et al., 2017). During DNA replication, the USP7 deubiquitinase can remove ubiquitin conjugated to SUMO, thus preventing degradation of sumoylated proteins at chromatin around replisomes (Lecona et al., 2016). Functional studies suggest that these mechanisms are important for DNA repair and replication (Hendriks et al., 2015; Lecona et al., 2016; Pfeiffer et al., 2017). SUMO removal from substrates by desumoylases provides another way of counteracting the effects of STUbLs. For example, the yeast Ulp2 desumoylase protects rDNA-bound sumoylated proteins from STUbL-mediated degradation, thus promoting ribosomal DNA stability (Gillies et al., 2016; Liang et al., 2017).

SUMO machinery localization is critical for regulating sumoylation levels.

Differential localization of SUMO pathway enzymes provides another means of controlling sumoylation levels (Fig 2B). For example, while the yeast Ulp2 desumoylase is mainly localized inside the nucleus with nucleolar enrichment, the other yeast desumoylase, Ulp1, is tethered to nuclear pore complexes (Panse et al., 2003; Srikumar et al., 2013). Ulp1 sequestration at the nuclear rim is important for sustaining global sumoylation levels and for processes sensitive to SUMO homeostasis, such as DNA repair and transcription (Palancade et al., 2007; Texari et al., 2013). Similarly, anchoring the Ulp1 homolog SENP1 at nuclear pore complexes is required for proper DNA repair (Chow et al., 2012; Duheron et al., 2017). SUMO E3s also show differential localization, such as the presence of the PML SUMO E3 at PML bodies, as described above.

Altering enzymatic localization is an effective mechanism for adjusting cellular sumoylation levels in response to environmental changes. A well-established example is the upregulation of sumoylation levels of many DNA repair and checkpoint proteins in yeast and human cells after genotoxic stress. This is achieved partly by targeting SUMO E2 and specific SUMO E3s to DNA damage sites (Cremona et al., 2012; Galanty et al., 2009; Morris et al., 2009;

Psakhye and Jentsch, 2012) via association between sumoylation enzymes and DNA lesion recognition complexes, including the ssDNA-binding complex RPA and DSB-binding MRX complex (Chen et al., 2016; Chung and Zhao, 2015). The SUMO pathway enzymes also change localization during the cell cycle. For example, when cells enter mitosis, the SUMO E3 PIASy and the SENP1 and SENP2 desumolyases are targeted to centromeric regions and kinetochores to orchestrate dynamic modifications required for mitotic progression (Cubenas-Potts et al., 2013; Ryu et al., 2010). Further investigation of enzymatic localization in additional situations and cell types will provide a fuller understanding of SUMO pathway regulation.

Modulation of the SUMO pathway enzymes by other protein modifications

Regulation of the SUMO machinery by other protein modifiers provides yet another strategy for altering sumoylation levels. For example, three other classes of modifications can induce large changes in SUMO E2 activity. In response to hypoxia, SUMO E2 acetylation reduces its interaction with sumoylation consensus sites, thus diminishing sumoylation of many proteins (Hsieh et al., 2013). On the other hand, SUMO E2 phosphorylation by CDK1/cyclin B during mitosis increases its overall activity toward forming thioester bonds with SUMO (Su et al., 2012; Wen et al., 2017). Finally, sumoylation of SUMO E2 favors SUMO chain formation in yeast and target discrimination in mammals (Klug et al., 2013; Knipscheer et al., 2008). Phosphorylation also exerts regulatory effects on other SUMO pathway enzymes. For example, Cdc5 phosphorylation of yeast Ulp2 desumoylase downregulates its function in mitosis, which is thought to preserve appropriate sumoylation levels required for mitosis (Baldwin et al., 2009). In another example, mTOR-mediated phosphorylation of SENP3 favors its interaction with nucleolar scaffold protein NPM1 to promote nucleolar targeting and SUMO2/3 removal at this location (Raman et al., 2014).

As outlined above, adjusting sumoylation levels on a large scale can be achieved by STUbLs and their antagonists, as well as by alteration of SUMO pathway enzyme localization and modifications. Such adjustment provides an economic means of simultaneously modulating multiple pathways, thus facilitating complex processes such as cell cycle progression and environment adaptation. We next review how the requisite tight integration of SUMO-based regulation with other signaling systems operating during the same cellular transitions is achieved.

Crosstalk between SUMO-based and other signaling processes

The above sections described specific situations in which the SUMO machinery is affected by other protein modifications and vice versa. On a larger scale, the physical and genetic interactions between sumoylation and signaling pathways mediated by other post-translational modifications remain to be explored for many contexts. Current progress on crosstalk between SUMO and phosphorylation- and ubiquitin-based signaling is summarized below.

Interactions with the DNA damage checkpoint pathways

Sumoylation and the DNA damage checkpoint pathway interact in multiple ways (Fig 3A). In both yeast and human cells, DNA damage-induced sumoylation and checkpoint-mediated phosphorylation occur in parallel but with significant substrate overlap (Cremona et al., 2012; Munk et al., 2017; Psakhye and Jentsch, 2012). These studies also show that changes in the checkpoint pathway can alter sumoylation events. In particular, reducing the function of the ATR checkpoint kinase or its cofactors leads to increased sumoylation of multiple proteins. Whether this reflects a direct role of checkpoint proteins in down-regulating sumoylation is not well understood, though the ATM checkpoint kinase can positively regulate SENP2 transcription in specific context (Lee et al., 2011). However, the opposite has also been reported, as ATM appears to promote sumoylation in the absence of ATR (Munk et al., 2017), suggesting context-dependent crosstalk.

Changing sumoylation levels also influences checkpoint functions. For example, sumoylation of ATR and its cofactor ATRIP in human cells supports genotoxin survival by boosting ATRIP interactions with other checkpoint factors and thus enhancing the checkpoint response (Wu et al., 2014; Wu and Zou, 2016). In contrast, ATM-mediated DNA damage checkpoint and cellular survival are promoted by transient inactivation of SUMO E1 and E2 via oxidative stress-induced disulfide bond formation between their catalytic cysteines (Stankovic-Valentin et al., 2016). These rather complex interactions between sumoylation and DNA damage checkpoint proteins under various conditions require further investigation to deconvolute the specific outcomes linked to distinct molecular mechanisms.

Interactions with other protein kinase-based signaling pathways

Recent proteomic data has helped to paint a more comprehensive picture of SUMO's functional interactions with protein kinases. Microarray-based assays found that a large number of kinases are sumoylated *in vitro* (Uzoma et al., 2018). Consistently, another proteomic study reported co-regulation of sumoylation and phosphorylation (Hendriks et al., 2017). In particular, sumoylated lysines are abundantly found near CDK1 consensus sites, and sumoylation of these sequences showed dependence on CDK1. This finding is in line with previously reported phosphorylation-dependent sumoylation (Hietakangas et al., 2006) and the aforementioned ability of CdK1 to enhance SUMO E2 activity (Su et al., 2012; Wen et al., 2017). One outcome of CDK1-mediated SUMO E2 regulation is that phosphorylated E2 interacts with the Polo-kinase, leading to the latter's SUMO1 conjugation. This in turn serves to promote Polo-kinase nuclear import and stabilization, driving mitotic progression (Wen et al., 2017). Such a kinase-SUMO E2-kinase regulatory relay has also been observed in other contexts (Fig 3B). In glioblastoma cells, CDKI-mediated phosphorylation of SUMO E2 promotes SUMO1 conjugation of CDK6, saving the latter from degradation by blocking its ubiqutination and consequently favoring G1-S phase transition and cancer development (Bellail et al., 2014). With the identification of a large number of substrates subject both to sumoylation and phosphorylation, additional modes of crosstalk between these modifications, as well as elucidation of their functional importance, are anticipated in the future.

Interactions with ubiquitination pathways

Hybrid SUMO-ubiquitin chains generated by STUbLs often lead to protein degradation, but they can also recruit proteins that recognize both ubiquitin and SUMO (Fig 3C). At DSB sites, such chains recruit the RAP80 scaffold protein that can preserve polyubiquitin chains as landing pads for recruitment of DNA repair proteins, such as 53BP1, that favor end joining DNA repair, and sequent recombination repair proteins, such as BRCA1 (Guzzo et al., 2012; Lombardi et al., 2017). Additionally, SUMO plays important roles in modulating multiple ubiquitin E3 functions in DSB repair (Figure 3C). For example, SUMO1 modification of BRCA1 can increase its ubiquitin E3 activity, while similar modification of HERC2 favors the formation of ubiquitin E2-E3 complexes composed of RNF8 and UBC13 (Danielsen et al., 2012; Morris et al., 2009). The combined effects of sumoylation and ubiquitination in these situations generally help recruit DNA repair factors and enhance their functions, but may also enable disassembly of repair complexes through STUbL or the Cdc48 segregase (Nie and Boddy, 2016; Schwertman et al., 2016). Sumoylation also regulates ubiquitination enzymes in other cellular contexts. For example, sumoylation of a subunit of the APC ubiquitin E3 in mitosis was recently suggested to help activate the latter for timely anaphase onset (Eifler et al., 2018; Lee et al., 2018). Of note, SUMO and ubiquitin can also antagonize each other's effects, sometimes via modification of the same site(s), as has been shown for DNA replication factor PCNA (Zilio et al., 2017).

Conclusion and outlook

A large body of work has established critical roles for sumoylation and desumoylation in many aspects of biology. While SUMO-mediated effects in specific processes have been reviewed, it is also useful to bring together findings from diverse arenas to derive common underlying regulatory mechanisms and identify the most significant roles of SUMO. By stepping back to take a broader perspective, the current body of knowledge offers a few take-home messages regarding the global effects of SUMO on nuclear function. First, as outlined above, dynamic sumoylation of multiple substrates can contribute to membraneless nuclear structures and functions therein. Similarly, the SUMO pathway and its many substrates serve necessary structural and regulatory functions at chromosome regions such as centromeres and heterochromatin. Moreover, sumoylation promotes DNA end movement and can directly affect transcription and DNA repair. Some of these events are a part of cellular response system entailing sumoylation induction of numerous proteins. The dynamic machinery localization. Finally, the SUMO pathway shows intricate interactions with kinase- and ubiquitin-mediated regulation and signaling processes such as during the DNA damage response and cell cycle progression.

Also outlined in this review are many questions that remain to be explored. For example, could SUMO:SIM interactions help to form a wide range of nuclear structures beyond PML bodies, and could these include centromere and heterochromatin domains? How can the SUMO system collaborates with nuclear actin and myosin to drive DNA movement? What are the mechanisms targeting SUMO pathway enzymes to specific sites, and how these can change during the cell cycle and upon changes in internal and external stimuli? What are the specific functional interactions between the SUMO system and the phosphorylation and

ubiquitination machinery in different contexts. Addressing these questions can be greatly facilitated by further evolution of techniques for studying sumoylation. For example, improvement of mass spectrometry-based detection of sumoylation sites can help to connect specific sumoylation events with unique biological outcomes. Mapping the complete set of sumoylation sites for low abundance proteins and directly monitoring sumoylated protein forms in tissues are still challenging. Another direction that awaits further development pertains to the understanding of sumoylation in human disease. Besides those mentioned above, such as acute promyelocytic leukemia and neurodegenerative diseases, the SUMO pathway is known to be related to tumorigenesis and host defenses against viral infections (Flotho and Melchior, 2013; Seeler and Dejean, 2017). The mechanism of SUMO-associated pathogenesis in many diseases is just beginning to be fleshed out, and a better understanding will pave the way for new diagnostic and treatment strategies aimed at modulating this dynamic post-translation modification.

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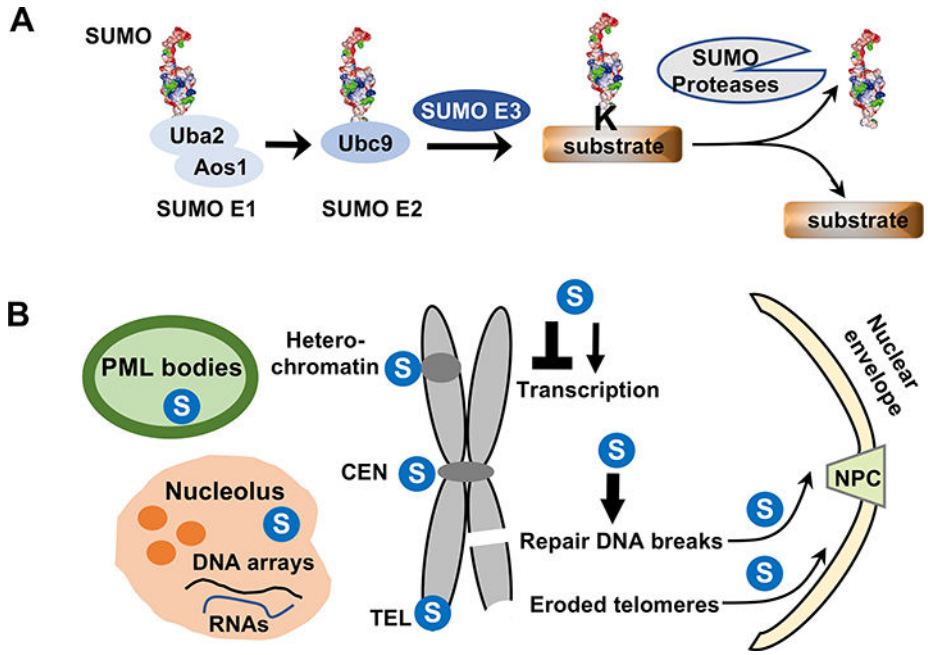

Figure 1: Dynamic SUMO conjugation cycle and its multiple effects on nuclear structure and functions.

A. Conjugation and deconjugation of SUMO (shown in 3D structure rendering) are outlined. SUMO forms a thioester bond with the heterodimeric E1 (Aos1/Uba2) in an ATP-dependent manner. SUMO is then transferred to the E2 (Ubc9), again forming a thioester bond. SUMO is conjugated to the lysine residue (K) on the substrate with the help of SUMO E3. Only a single SUMO conjugation is shown, but multiple SUMOs or SUMO chains can also be found on substrates. SUMO proteases cleave SUMO from the substrate.

B. A brief summary of major effects of sumoylation on nuclear structure and functions. Nuclear domains and chromosomal regions enriched with SUMO are indicated by S. Sumoylation also regulates transcription and DNA lesion repair as indicated. Arrow: positive effects; lines: negative effects. Arrows pointing to the nuclear pore complex (NPC) and nuclear envelope indicate SUMO-mediated DNA movement toward these locations.

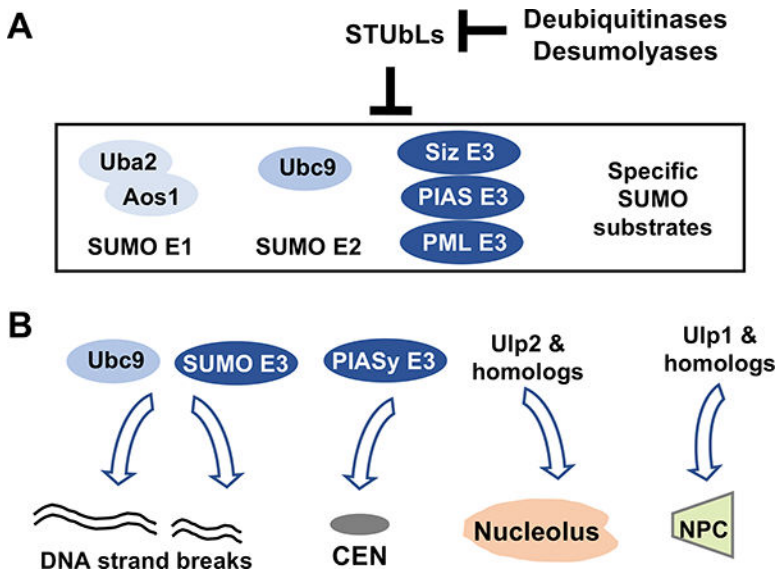

Figure 2: Examples of SUMO pathway regulation.

A. SUMO E1, E2, multiple SUMO E3s, and specific SUMO substrates can be targeted by STUbL-mediated protein degradation, an effect that can be counteracted by specific deubiquitinases or desumoylases.

B. SUMO E2, SUMO E3s, and desumoylases can be targeted to specific nuclear and chromosomal structures to induce large scale changes in sumoylation.

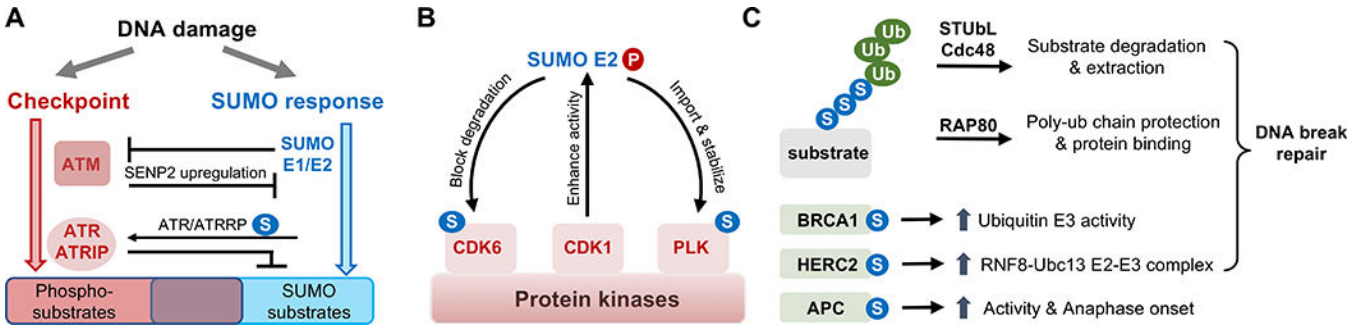

Figure 3: Examples of crosstalk between SUMO-based regulation and other signaling processes

A. Interactions with the DNA damage checkpoint pathways. As described in the text, genome stress such as those caused by genotoxin treatment can induce a SUMO-based response and the ATM/ATR checkpoint kinase-mediated response. The two responses are independent but overlapping, and exhibit context-dependent and multi-layered genetic interactions. The enzymes in the two pathways show both positive (arrows) and negative (lines) genetic interactions as summarized in the text. In addition, they can target a group of common substrates, which provide another layer of crosstalk.

B. SUMO E2 interactions with protein kinases. As described in the text, CDK1-mediated phosphorylation of SUMO E2 can increases E2 activity. Phosphorylated SUMO E2 can promote the sumoylation of the polo-kinase (PLK) and CDK6 in different cellular contexts. These sumoylation events can positively influence substrate kinase functions.

C. Crosstalk with ubiquitination pathways. As detailed in the text, the hybrid SUMO-ubiquitin chain generated by STUbLs can lead to protein degradation or Cdc48 segregase-mediated protein extraction, but may also result in recruitment of proteins like RAP80, which recognize hybrid chains. RAP80 recruits additional proteins to DNA breaks and favors end-joining repair. SUMO can also modify ubiquitin ligases, such BRCA1, HERC2, and APC. Their sumoylation promotes specific activities or scaffolding roles, as depicted. Several types of this regulation occur during DSB repair.