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Morris, Joanna R; Garvin, Alexander J

*DOI:*
10.1016/j.jmb.2017.05.012

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*Document Version*
Peer reviewed version

*Citation for published version (Harvard):*
Morris, JR & Garvin, AJ 2017, 'SUMO in the DNA Double-Stranded Break Response: Similarities, Differences, and Cooperation with Ubiquitin', *Journal of Molecular Biology*, vol. 429, no. 22, pp. 3376-3387. https://doi.org/10.1016/j.jmb.2017.05.012

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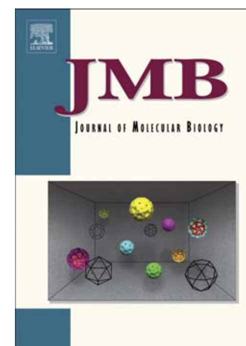


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Joanna R. Morris, Alexander J. Garvin

PII: S0022-2836(17)30227-9
DOI: doi:10.1016/j.jmb.2017.05.012
Reference: YJMBI 65414

To appear in: *Journal of Molecular Biology*

Received date: 17 March 2017
Revised date: 12 May 2017
Accepted date: 12 May 2017

Please cite this article as: Morris, J.R. & Garvin, A.J., SUMO in the DNA Double-Stranded Break Response: Similarities, Differences and Co-operation with Ubiquitin, *Journal of Molecular Biology* (2017), doi:10.1016/j.jmb.2017.05.012

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# SUMO in the DNA Double-Stranded Break Response: Similarities, Differences and Co-operation with Ubiquitin.

Joanna R. Morris and Alexander J. Garvin

*Birmingham Centre for Genome Biology and Institute of Cancer and Genomics, Medical and Dental School, University of Birmingham, Edgbaston, B15 2TT, UK.*

**Abstract**

In recent years our knowledge of the varied role that ubiquitination plays in promoting signal amplification, novel protein interactions and protein turn-over has progressed rapidly. This is particularly remarkable in the examination of how DNA Double-Strand Breaks (DSBs) are repaired, with many components of the ubiquitin conjugation, de-conjugation and recognition machinery now identified as key factors in DSB repair. In addition, a member of the ubiquitin-like family, SUMO (Small Ubiquitin-like Modifier) has also been recognised as integral for efficient repair. Here we summarise our emerging understanding of SUMOylation both as a distinct modification and as a co-operative modification with ubiquitin, using the cellular response to DNA double-strand breaks as the primary setting to compare these modifications.

## SUMO and Ub share similar conjugation enzyme architectures

Ubiquitin (Ub) and SUMO are small, globular proteins, produced as inactive precursors that require processing to generate mature proteins. Ub and SUMO have similar conjugation pathways. E1 activating enzymes charge C terminal glycine residues, E2 conjugating enzymes then transfer the Ub or SUMO from the E1 to the substrate via an intermediary cysteine residue. E3 ligating enzymes aid in substrate recognition and help guide the Ub or SUMO conjugate to target lysines. Each conjugating cascade is specific for their respective modifier [1, 2]. The SUMO conjugation system features far fewer components than the Ub system, with only a single E2 (Ubc9) versus the ~40 conjugation enzymes for Ub and a dozen or so known SUMO E3 enzymes compared to hundreds of ligases for Ub [3-5]. SUMO conjugation sites differs from ubiquitin conjugation sites in that ~75% of SUMOylated lysines occur within a consensus sequence "ψKxE/D" or its various derivatives (where 'ψ' is a large hydrophobic residue), while ubiquitination shows little preference for lysine context [6, 7]. This may be due to the large number of E2/E3 enzyme pairs in the Ub conjugation system compared to the single E2 for SUMO. Also unique to Ubc9, is the ability to directly conjugate SUMO to substrates without E3 enzymes so Ubc9 alone can dictate SUMOylation consensus lysine targeting [8]. In the DSB repair response the SUMO E1, E2 and E3 enzymes have been detected at sites of damage suggesting the SUMO conjugation machinery is locally recruited [9-11].

## SUMO is a family of related modifiers

SUMO and Ub are each encoded by several genes, but the matured protein products of Ub genes are identical, whereas SUMO genes (SUMO1-4) give rise to distinct protein products. The tertiary structures of SUMO and Ub proteins are similar, but they share limited sequence identity and differ in their surface charge distribution [12]. SUMO1 was first

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identified as an Ub-like protein that interacted with RAD51 and RAD52, localised to PML bodies, and conjugated to the GTPase RanGAP1 [13-16].

SUMO2 and SUMO3 share the same conjugation machinery to SUMO1 but are more distantly related [4, 5]. The 97% identity between SUMO2 and SUMO3 and the inability of antibodies to discriminate between them has lead to their collective designation as SUMO2/3. However some differences have been noted, SUMO3 contains a phosphorylation site ($Ser^2$) not found in SUMO2 [17, 18] and the differences in the C terminal extensions affect maturation efficiency *in vitro* [19, 20]. The SUMO2 and SUMO3 genes show distinct transcriptional responses to oxidative stress, and over-expression of SUMO3 but not SUMO2 regulates turnover of the Flap structure-specific endonuclease, FEN1 [21, 22]. To add to the difficulty distinguishing between these modifications there is inconsistency between research groups concerning the naming of SUMO2 and SUMO3 and often-times their designations are inverted. Wherever possible in this review we have used SUMO2 = P61956 and SUMO3 = P55854.

SUMO4 is homologous to SUMO2 but processing to its mature form is restricted by a proline residue within the C terminal tail [23]. Nevertheless SUMO4 may be processed by other hydrolases in response to stress [24], so that the cellular role for SUMO4 remains to be determined. SUMO5 (*SUMO1P1*) has recently been described as a regulator of PML bodies with restricted tissue expression [25]. The human genome contains many putative SUMO pseudogenes which include *SUMO5/SUMO1P1* [26] and further work is needed to ensure SUMO5 is a translated, endogenous protein. Thus unlike ubiquitination, which represents a single modification, SUMOylation encompasses several distinct modifications that share common conjugation machinery (Fig 1).

**Paralog specific SUMOylation and deSUMOylaation**

SUMO1 and SUMO2/3 modifications can be entirely distinct and some proteins are preferentially modified by particular paralogs while others can be modified by both [27]. The availability of different SUMO family members to the conjugation machinery may influence SUMO paralog specific modifications. A significant fraction of SUMO1 is localised to nuclear pores as SUMO1-RanGAP1 conjugates, while SUMO2 and SUMO3 pools are less spatially restricted. This may explain the greater dynamic alterations in SUMO2/3 versus SUMO1 conjugation that occurs in response to cellular stress [28, 29].

The paralog specificity of certain target protein modifications can be influenced by the activity of SUMO protease enzymes which possess paralog editing specificity. Six SUMO proteases (SENP1-3 and 5-7) and three additional enzymes (DeSi1/2 and USPL1) are able to cleave SUMO from substrates [30-32]. Maturation of pro-SUMO to the mature form that terminates at a glycine is carried out by SENP1 and SENP2 [33]. SUMO1 deconjugation is predominantly carried out by SENP1 and to a lesser degree by SENP2 [34], while SUMO2/3 modifications can be removed by all SENPs [33, 35]. SENP6 and SENP7 are specialised SUMO2/3 chain depolymerising enzymes [36, 37]. It is possible that the relative paucity of SUMO1-cleaving proteases may explain why SUMO1 modification of substrates appears more pronounced than SUMO2/3 conjugation when the isoforms are over-expressed - the artificial SUMO1 levels overwhelm limited cellular deconjugation, while more abundant SUMO2/3 proteases can better compensate for artificially elevated SUMO2/3 [38]. In

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addition, SUMO2/3 modification in the form of polymers can trigger degradation of some substrates which may limit the detection of highly SUMO2/3 modified proteins (discussed later) [39, 40]. Paralog specificity may also be enforced at the level of localisation as SUMO deconjugating enzymes distribute to distinct sub-cellular locations, for example, SENP2 localises to nuclear pores [41], SENP3/5 to nucleoli [42], SENP7 to chromatin [43-45] and USPL1 to Cajal bodies [32].

Some degree of redundancy between SUMO1 and SUMO2/3 may be tolerated in cells as mice deficient for SUMO1 are viable due to compensation by SUMO2/3 [46, 47]. Similarly compensation by SUMO2 rescues the lethality of SUMO3 deficient mice [48]. In contrast SUMO2 loss is embryonic lethal, which is thought to be due to inadequate compensation from the less abundant SUMO3 protein during development [48]. Therefore, at least in the context of mouse embryonic development SUMO1 is redundant with SUMO2/3 but not *vice versa.*

**SUMO forms multiple types of modification**

SUMO conjugates, like Ub conjugates, can occur in the form of mono, multi-mono, polymers and branched chains. SUMO polymers are generated by SUMOylation of an internal lysine (K11) embedded within a consensus site in SUMO2/3 [49]. Intriguingly although K11 linkages of SUMO2/3 are the most abundant (~60% of SUMO2-SUMO2/3), linkages have also been detected on many other SUMO2/3 lysine residues [6, 7, 50], suggesting distinct SUMO chain types exist. Additionally, peptides belonging to SUMO2 branched chains in which SUMO2 is doubly modified at K7 and K11 or K5 and K7 have been detected [51]. The discovery of differing linkages of Ub has increased our understanding of the complexity of Ub signalling substantially [52, 53]. SUMO2/3 polymers may also have highly complex structures with potentially diverse cellular functions.

The lack of SUMO consensus conjugation site on SUMO1 suggests that it is less likely to form chains *in vivo* [49]. However SUMO conjugation site mapping has revealed that SUMO2 is conjugated to SUMO1 at several lysine residues and that SUMO1-SUMO2/3 mixed linkages occur in cells [6, 7, 50]. SUMO1 incorporation into SUMO2/3 polymers may act as a capping mechanism, preventing further chain elongation [18]. Alternatively, given the multiple SUMO1-SUMO2 pairs identified on non-consensus SUMO1 lysines [6, 7, 50, 51], it is also possible these chains are more complex (Fig 2).

**Mixed SUMO~Ub polymers**

The complexity of SUMO polymers is increased further by the discovery of mixed SUMO-Ub polymers. SUMO1 and SUMO2/3 are ubiquitinated at several lysine residues, most likely by SUMO targeting Ub E3 ligases such as RNF4 and RNF111 [39, 40, 54]. In addition Ub is extensively modified by SUMO2, suggesting both ubiquitination of SUMO and SUMOylation of Ub [50]. SUMO2/3 is conjugated to different Ub lysines depending on the condition, switching from K63 to K11 residues upon proteotoxic stress [7]. Incorporation of SUMO2/3 into ubiquitin polymers at sites such as K63 would presumably impact the cells ability to signal through this type of ubiquitin linkage, generating entirely new chain topologies for

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other binding domains. Therefore this crosstalk between Ub and SUMO could substantially increase the complexity of small modifier recognition in cells [7].

**SUMO and Ub act as docking sites mediating protein-protein interactions**

A major function of small modifier conjugation such as Ub and SUMO is the additional interaction interfaces they provide to their modified substrates. This allows docking of protein domains that would otherwise not occur on unmodified substrates. More than 20 Ub interaction domains have been identified, some of which display preferences for monomers or for specific chain types [55]. In contrast until recently the only known SUMO binding elements were the SUMO Interacting Motifs (SIMs). These are short sections of small hydrophobic amino acids with similarities to the sequence (V/I/L)x(V/I/L)(V/I/L), which form a hydrophobic core within a groove on the surface of SUMO [56]. Local amino acid charge variation, in particular acidic or phosphorylated residues can determine specificity in binding SUMO paralogs [56, 57], [58-60]. SIMs are present in the conjugation machinery enzymes, and for some may assist SUMO conjugation activity [61, 62]. SIM motifs in substrates can also promote paralog specific modification [63]. Recently SIMs in the N terminus of the SUMO E3 ligase ZNF451 were shown to be required for SUMO2/3 chain forming ability and for SUMO2/3 preference [64, 65]. Additional binding surfaces on SUMO distinct from the patch recognised by SIMs include the "backside" patch that interacts with Ubc9 and the E67 loop that interacts with the protease DPP9 [66, 67].

In addition to SIMs, two more SUMO interacting domains have been identified, the zinc finger MYM, and ZZ domains. The MYM domain binds the same SUMO surface as SIM motifs, while the ZZ domain contacts a different surface and has a preference for binding SUMO1 over SUMO2/3 [68-70]. Interestingly the MYM domain containing protein ZMYM3 has recently been described as a factor required for recruiting BRCA1 to sites of damage, although it is not known if its SUMO binding activity is required for this function [71].

**SUMOylation can promote Ub-dependent clearance**

Polyubiquitin conjugation often serves as degradation signal, directing proteins for proteasome mediated degradation [72], however the multifarious functions of Ub have expanded as our view of the modification in signalling has advanced [3]. Conversely, our perspectives of SUMOylation as a non-proteolytic signalling molecule have also shifted. Cells treated with the proteasome inhibitor MG132 rapidly accumulate high molecular weight Ub adducts due to failure of the proteasome to clear ubiquitinated proteins, in addition they also accumulate SUMO conjugates [73]. This suggested that both modifications are processed by proteasomal degradation. It is now clear that specialised E3 Ub enzymes such as RNF4 and RNF111 [39, 40, 54], recognise and ubiquitinate SUMO2/3 polymers through SIM motifs providing insight into how SUMOylation can direct proteins for degradation [39, 40].

**The Setting: Double-Stranded Break Repair**

DNA double-stranded breaks (DSBs) are highly toxic lesions. These breakages need to be rapidly repaired to prevent loss of genetic data, chromosome fusions and ultimately cell death [74]. Improper DSB repair results in genomic instability linked to cancer, aging and immune dysfunction. Ionising radiation (IR), various chemotherapies and stalled replication

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forks can promote the formation of DSBs. Their repair involves the localised recruitment of DNA repair sensors and effecter proteins to sites of damaged chromatin. In mammals the majority of DSBs are repaired by ligating the broken ends together in a process termed Non-homologous end joining (NHEJ). The processing of the broken ends during NHEJ often leads to loss of DNA and is therefore mutagenic. If DSBs form in S/G2 phases of the cell cycle when a second copy of the DNA is available as template, cells can utilise the main homology repair (HR) mechanism of gene conversion, which uses resection of the DNA surrounding the break followed by homology searching by RAD51 recombinase. DNA is faithfully repaired without any changes in genetic material (repair pathways are reviewed in detail elsewhere [74]. Many factors involved in DSB repair are SUMOylated in both basal conditions and in response to genotoxic stresses [75, 76], the role SUMOylation plays in regulating those proteins repair activities is beginning to emerge.

**Paralog specific SUMOylation and deSUMOylation in the DSB response.**

In the DSB repair response both SUMO1 and SUMO2/3 modifications occur on damaged chromatin as detected by ionising radiation induced foci (IRIF), recruitment to laser-induced DNA damage lines, recruitment to DSB flanking Lac-operon arrays, and enrichment detected by ChIP near to break sites [9-11, 75, 77-79]. Some reports have also identified distinct recruitment kinetics for SUMO1 versus SUMO2/3 in their enrichment to sites of damage [80, 81] and DSB signalling promotes paralog specific SUMOylation in some repair factors. Factors that accumulate or are modified early in the response such as γH2AX (H2AX phosphorylated at $Ser^{139}$), MDC1, HERC2 and RNF168 are predominantly modified by SUMO1 [69, 82, 83] while later factors such as BRCA1, 53BP1 and EXO1 can be modified by both SUMO1 and SUMO2/3 [9] [10, 84]. These temporal differences in SUMO paralog modification may explain the differing kinetics of SUMO conjugates detected at DSBs [80, 81]. The preferences for paralog conjugation could be due to the activities of two SUMO E3 enzymes that localise to DSBs. PIAS4 is required for the earlier phases of repair, upon which SUMO1 modification appears critical whereas PIAS1 is required for SUMO2/3ylation of later arriving components [10, 69, 82]. It is not yet clear how paralog specific conjugation arises from these E3 ligases.

The redundancy observed in mouse development between SUMO1 and SUMO2/3 may not be reflected in DSB repair as U2OS cells depleted of SUMO2/3 are radiosensitive, suggesting SUMO1 cannot compensate for SUMO2/3 loss [75]. In I-*SceI* DSB reporter assays SUMO1 depletion has a more profound effect on repair efficiency than SUMO2/3 depletion suggesting both forms of SUMOylation are required for proper DSB repair [85].

Our understanding of the potential roles for cellular SUMO chains is hampered by a lack of specific reagents and currently it is not possible to discriminate between SUMO2/3 multi-mono conjugates *Vs* SUMO polymers *in vivo*. In the ubiquitin field a growing number of reagents, such as antibodies that detect Ub conjugates and distinct Ub chain types (K48, K63 and K11), or specific Ub chain type sensors have informed the role that Ub plays in DNA repair [86-89]. While improved SUMO reagents are on the horizon the presence of SUMO polymers, or possibly multi-mono conjugates can be inferred by the recruitment of SUMO specific Ub E3 ligases, such as RNF4 to DSBs. In these proteins SIMs (SUMO Interacting motifs) are arranged in tandem to allow reading of poly or multi SUMO2/3 modifications. Mutation of RNF4 SIMs abrogates recruitment to DSBs suggesting the presence of

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polymers/multi-monomers [39, 40, 75, 77-79]. Our understanding of the biological significance of multi-mono modifications by SUMO is also poorly understood. A screen for proteins that interact with a multi-mono SUMO2 mimic identified several proteins that have roles in DSB repair, including BLM and 53BP1 [91]. The presence of multiple SUMO conjugates on a substrate may aid in co-ordinating protein-protein interactions between multi subunit protein complexes and remains to be studied in greater detail.

**SUMO and Ub deconjugation are essential for proper DSB repair**

The editing function of DUBs (De-ubiquitinating enzymes) are an essential component of DSB signalling acting as critical nodes to prevent over-accumulation of Ub signals and balance modification of repair factors [92-96]. The SUMO system has relatively few SUMO proteases. Consequently SENP enzymes are less able to compensate for one another during the DSB repair response. Indeed depletion of each SENP (except SENP3) individually alter HR and NHEJ repair efficiencies in *ISceI* reporter assays [45]. To date only the two chain editing SENPs have been studied for their roles in DSB repair. SENP6 interacts with RPA70, a protein that coats single-stranded DNA generated during replication and following DNA resection during HR repair. SENP6 maintains RPA70 in a hypoSUMOylated state, but following replication stress, dissociates from RPA70 allowing SUMOylation to proceed. RPA70 SUMOylation is required for the loading of RAD51 - the subsequent step required for homology search and successful gene conversion repair [90]. SENP7 is also required for HR repair through constitutive deSUMOylation of the transcriptional repressor and heterochromatin component KAP1/TRIM28. Phosphorylation of KAP1 by ATM during DSB repair weakens the SUMO dependent interaction between it and the NuRD (Nucleosome Remodelling Deacetylase complex) subunit CHD3 (Chromodomain Helicase 3) [97]. This allows release of the chromatin condensing activities imposed by the NuRD complex which impede DSB repair. Loss of SENP7 promotes hyperSUMOylation of KAP1 and prevents CHD3-NuRD eviction and the chromatin remodelling that occur in response to DSBs, resulting in downstream failure to generate RAD51 filaments and subsequent repair [45].

Therefore, just as with DUBs, deSUMOylases are critical for managing the steady state SUMOylation of factors that are employed during DSB repair and in facilitating rapid changes in SUMOylation status in response to stress stimuli.

Ubiquitin and SUMO chains are each depolymerised by specific proteases, but the identity of enzymes that recognise mixed linkages is not well understood. DUB enzymes USP11 and USP7 have been proposed to act on hybrid chains via disassembly of the Ub component [98, 99]. It is not clear if they have any intrinsic specificity for mixed chains or simply remove ubiquitin from SUMO as they would many other substrates. Interestingly both proteins have important roles in genome stability, though their specific role in removing Ub from SUMO chains is not yet clearly associated with those roles [98-104]. The UCH type DUB UCH-L3 can also cleave Ub conjugated to SUMO2 at K11, and from artificial linear SUMO2 chains *in vitro* [105]. In cells UCH-L3 is recruited to sites of damage where its activity promotes the interaction between RAD51 and BRCA2 [95, 106]. Whether this role relates to regulation of Ub~SUMO polymers is not yet known.

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**SUMO and Ub act as docking sites mediating protein-protein interactions in the DSB response.**

**SUMOylation regulates recruitment of the Ub machinery at DSBs**

The importance of Ub as nucleating factor in the DSB repair response is well documented (for reviews [107-109]). Ubiquitin signalling in DSB repair is promoted by a sequential relay of E3 Ub ligases. SUMO has an extensive impact on the sequential ordering of the cellular response to DSBs and directs both ubiquitin conjugation and deconjugation within the DSB repair pathway [9, 10, 75, 77].

HERC2 promotes the activity of the first Ub ligase associated with DSBs, RNF8, via interaction with the K63 specific E2 enzyme Ubc13 [110]. HERC2 is SUMOylated in response to IR and it has been suggested this promotes intramolecular re-organisation to further enhance its ability to activate the E3 Ub ligase activity of RNF8. K63-Ub conjugates generated by RNF8 promote recruitment of another Ub ligase, RNF168 [69, 89]. The PIAS4 SUMO ligase regulates transcription of RNF168, and RNF168 is SUMOylated in response to IR [69]. Moreover RNF168 interacts with SUMO2-K63-Ub mixed chains [111] suggesting SUMO may have several influences on RNF168-mediated signalling. The ubiquitinated product of RNF168 ligase activity (Histone H2A and H2AX ubiquitinated at K13/15) recruits 53BP1 - a scaffold protein that antagonises the ability of BRCA1 to promote resection in homologous recombination [112, 113]. 53BP1 is also SUMOylated during DSB repair, but what function this has is currently unknown [10, 75].

Ub-chains act as a recruitment scaffold for the BRCA1-A complex component RAP80. Ubiquitin interaction motifs (UIMs) in RAP80 recognise K63-Ub linkages enabling the recruitment of BRCA1-A [114-117]. More recently RAP80 has been identified as a dual K63-Ub SUMO2 interacting partner via a SIM motif adjacent to the UIMs. The SIM is needed for full recruitment of RAP80 and BRCA1 to DSBs [80, 118, 119]. RAP80, along with other BRCA1-A components BRCC36, Abraxas, BRE and MERIT40 have been detected as binding partners of mixed SUMO2~K63 Ub chains in cell lysates [111] and RNF4, which can generate K63 linked Ub~SUMO2 mixed chains *in vitro* [120] could serve as a source for this signal [118]. RNF4 is also an additional example of an Ub E3 ligase recruited to DSBs via SUMO [75, 77, 78]. SUMO2/3 also regulates RNF4 activity by SIM directed dimerisation and ultimately degradation through auto-ubiquitination [121].

BRCA1 is SUMOylated in response to various DNA damaging agents [9, 10, 50] and SUMOylation enhances its Ub ligase activity *in vitro* [9]. The BRCA1:BARD1 Ub ligase activity modifies lysines in the extreme C terminus of H2A (K125/127/129) [122] and in DSB repair the BRCA1 E3 ligase function stimulates 53BP1 re-positioning and DNA resection required for HR through promoting the recruitment and activity of the chromatin remodeller SMARCAD1 [123]. Whether SUMOylation potentiates this activity is not yet clear.

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CBX4 is a SUMO E3 ligase within the Polycomb repressor complex 1 (PRC1) that is recruited to DSBs [81]. IR promotes CBX4 dependent SUMOylation of BMI-1 which is essential for BMI-1 localisation to sites of damage. Together with RING1a/b, BMI-1 has E3 Ub ligase activity responsible for the majority of Ub modified H2A (at K118/K119) in cells. In addition to a general role in transcriptional repression at promoters this modification and BMI-1/RING1b have been implicated in further Ub-signalling at DNA double-strand breaks and transcriptional repression local to DSBs directed by the repair response [124-126].

**SUMOylation dependent regulation of DUB recruitment and activity**

SUMO also aids recruitment of the DUB enzyme Ataxin 3 (ATXN3). DSB localisation of ATXN3 is rapid and dependent on SIM interaction with SUMO1, but is independent of Ub binding via its UIM motifs. ATXN3 is required for accumulation of RNF8, RNF168, ubiquitin, 53BP1 and BRCA1 to sites of damage and thus for efficient HR and NHEJ repair [95] [79].

DUB enzyme activity can be regulated by both ubiquitin [127] and SUMO. SUMOylation of USP28 at K99 or direct fusion of SUMO2 to USP28 inhibits its DUB activity [128]. It is not known what impact this has in cells but USP28 is an interacting partner of 53BP1 that modulates 53BP1's transcriptional activity towards p53 [95, 129, 130]. In addition to the SUMO dependent recruitment of ATXN3, free SUMO1 can also stimulate ATXN3 DUB activity against Ub-K63 chain *in vitro.* Thus non-covalent interactions with SUMO may also regulate DUB catalytic function [79].

**SUMOylation regulates recruitment of repair factors at DSBs**

SLX4 is a scaffold protein that recruits nucleases to DNA lesions in various contexts [131]. SLX4 binds SUMO through SIM motifs in addition to Ub via UBZ domains [132-134]. The SIMs in SLX4 are required for recruitment to laser induced damage and collapsed replication forks, whereas Ub binding, but not the SIMs, is required for its accumulation at inter-strand cross links. Thus recognition of SUMO and Ub independently can channel SLX4 into different repair structures [132, 133, 135].

The helicase BLM has roles in both promoting and preventing RAD51 dependent recombination and is extensively SUMOylated at multiple sites [50, 136]. Mutation of two SUMOylation sites in BLM promote its localisation to IRIF rather than PML bodies, suggesting that SUMOylation may function to prevent BLM from accessing DSBs by sequestration into PML bodies [136]. RAD51, while not SUMOylated itself interacts with SUMO via a conserved SIM. Indeed SUMO1 was first identified through a yeast two hybrid screen for RAD51/RAD52 interactors [13]. Mutation of the RAD51 SIM disrupts filament formation and HR repair suggesting SUMO is essential for the homology search component of HR, but the SUMOylated factors to which RAD51 interacts are currently unknown [11].

**SUMO acts as a scaffold for protein group recruitment in the DDR.**

In 2012 the Jentsch group proposed the concept that SIM:SUMO interactions promote protein group modification [137]. In this process entire complexes, and perhaps super-complexes, are SUMOylated. Interaction between these SUMOylated components with near-by proteins and other components bearing SIMs then promotes the complex and

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super-complex interaction. In yeast localisation of SUMO E3 ligase, Siz1, to DSBs occurs via its DNA binding SAP domain and through interaction with the critical DSB sensor complex, MRX (MRE11/RAD50/Xrs2). The ligase then promotes modification of multiple repair factors proximal to sites of damage. Their collective modification and interactions, rather than individual modifications, is then responsible for repair. The group modification hypothesis is supported by proteomics analysis of SUMOylation substrates where frequently all members of some protein complexes are SUMO modified [50], and it explains why so often researchers find that mutation of single SUMO conjugation sites in target proteins have only mild effects on investigated phenotypes [137, 138] .

It is not yet clear how significant this group modification is in the mammalian double-strand break response where multiple SUMO isoforms, ligases and deSUMOylating enzymes add additional complexity to SUMOylation outcomes (Reviewed in Garvin and Morris Phs Trans B 2017). One potential example of group modification concerns the ATR kinase partner ATRIP. SUMOylation of ATRIP has a mild impact on function but regulates interaction with multiple binding partners, suggesting this modification is more important for complex formation than direct activity of ATRIP [138].

**SUMO promotes Ub dependent clearance of DSB repair factors.**

SUMO conjugates present at sites of damage interact with the tandem SIM domains of the Ub ligase RNF4 and direct its rapid recruitment [75, 77-79, 82]. RNF4 promotes the clearance of the DSB repair scaffold protein MDC1 from sites of damage and its removal is critical to subsequent repair steps [75, 77, 82]. RNF4 activity has also been implicated in the proteasomal clearance of BRCA1, 53BP1, BLM, RPA, EXO1 and KAP1 suggesting multiple points in DSB repair in which RNF4 is required [77, 78, 84, 139, 140]. Thus SUMO modification and subsequent interaction with RNF4 is essential for the step-wise progression of the repair process.

Some ubiquitinated proteins are physically removed from their surroundings by the AAA ATPase complex VCP/p97, often prior to their proteasomal degradation. It can act on nuclear proteins in the context of chromatin and replication forks [141-143]. VCP and its adapters are recruited to DSBs through interactions with ubiquitin [144, 145]. In yeast dual SUMO/Ub recognition by a VCP adapter is essential for DSB repair [146-148] and may have a similar activity in mammalian cells [147].

**SUMO-like domains and Ub-like domains**

Protein domains that mimic the structure of Ub are important for regulating the activity of components of the Ub enzyme pathway [149]. In yeast SUMO like domains (SLD) in RAD60 are important for recombination after replication fork collapse through an unknown mechanism possibly involving an interaction with Ubc9 to modulate SUMO chain formation, target specific substrates for SUMOylation, or attenuate of Ubc9 activity [150, 151]. SLDs in mammalian cells are found in UAF1, a heterodimeric partner of several DUB enzymes. UAF1 contains two SLDs that interact with SIMs in RAD51AP1 and FANCI [152, 153]. These domains mediate the formation of a ternary complex (UAF1-RAD51-RAD51AP1) required for recombinase activity and homologous recombination [153].

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**Expanding complexity in SUMO and Ub signalling**

This review has focussed primarily on two modifications, but our growing understanding of post-translational signalling suggests a far more complex story. The modifiers themselves, SUMO and Ub, can be subjected to post translational regulation; acetylation of SUMO1 and SUMO2 neutralises positively charged lysine residues required for interaction with some SIMs, and can provide additional protein interaction faces to acetyl reading domains such as the bromodomain of p300 [154, 155]. The dominant chain forming lysine residue (K11) in SUMO2/3 can also be acetylated [156] suggesting the possibility that acetylation/deacetylation regulates SUMO chain formation. Mixed linkages between SUMO and NEDD8 and NEDD8 and Ub have been described adding more complexity to the system [7], [157]. Possibly the enzyme RNF111/Arcadia, described as a SUMO targeted Ubiquitin ligase [54, 158, 159], can also generate NEDD8 polymers in this context [160].

Phosphorylation through the apical repair kinases ATM and ATR plays an essential part in DSB signalling [161]. Kinases required for cell cycle progression such as CDKs are also important in the repair processes as these links DSB sensing to checkpoint arrest and correct repair pathway choice. Recently CDK-mediated phosphorylation has been associated with SUMO site modification in a sub-set of substrates [7]. Phosphorylation can also alter SUMO-SIM interactions, and modify SUMO isoforms themselves [60]. Since the complexity of the Ub code, mediated by different chain topologies and by post translational modification of Ub such as phosphorylation can trigger specific cellular responses [53, 162-164], it seems likely that SUMO isoforms, chains and modifications convey a wide range of signals in a similar fashion.

**Conclusion**

In the last decade it has become apparent that Ub signalling in the DSB response is exquisitely complex and contributes to the integration of cell cycle stage and chromatin state with correct pathway choice. Almost a decade since the first identification of SUMO recruitment to DSBs our knowledge of SUMOs role in repair has moved on, but has still lagged behind its more glamorous cousin ubiquitin. However deepening our understanding of SUMO and its complexity alone will not be sufficient. SUMO, Ub and other PTMs are so extensively intertwined in DSB repair that significant insights will come from studying these proteins as co-operative modifiers. Given the importance of DSB repair in pathways relevant to health and disease such an approach will help accelerate development of therapeutic approaches.

**Acknowledgments**

AG is funded by CRUK (C8820/A19062) and JRM by the University of Birmingham.

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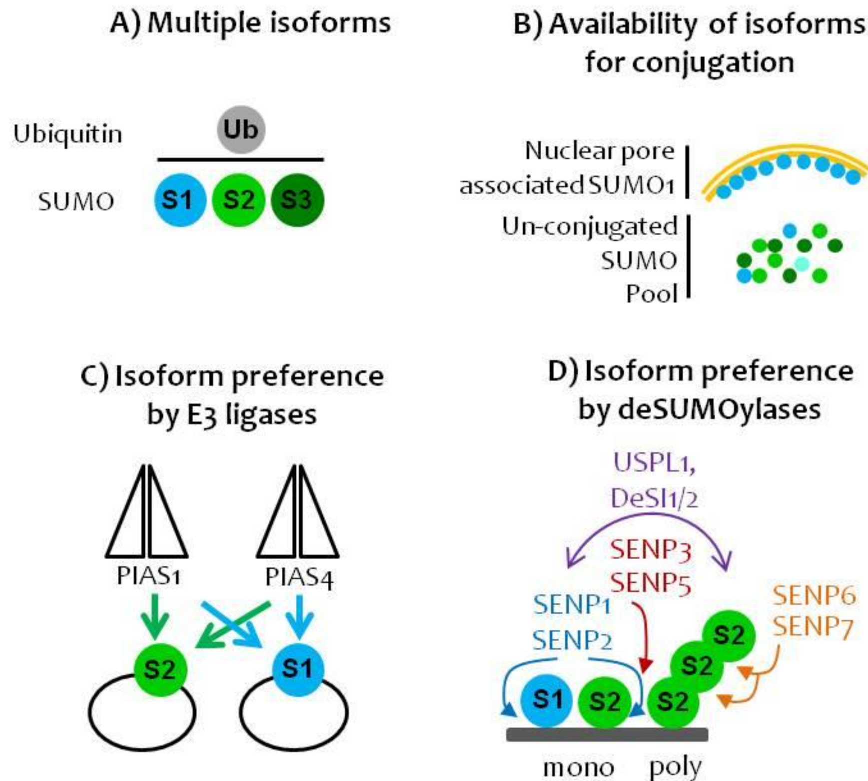


**Figure 1. Paralog specific SUMOylation.**

A) SUMO is composed of at least 3 mature isoforms. SUMO4 and SUMO5 are omitted here as they may not be involved in conjugation. B) Availability of free pools of SUMO1 and SUMO2/3 may influence paralog dependent SUMOylation. SUMO1 is enriched at nuclear pores in the form of SUMO1 modified RanGAP1 while SUMO2/3 are less restricted in their localisation. Additionally a larger proportion of SUMO1 is found in conjugated pools compared to SUMO2/3. C) SUMO E3 ligases can promote paralog specific SUMOylation of some substrates - though the mechanisms are not well understood. D) SENP proteases exhibit some degree of paralog specificity and preference for SUMO polymers over monomers. The activity of SENP enzyme is likely important for defining paralog specific modification of SUMOylation substrates.

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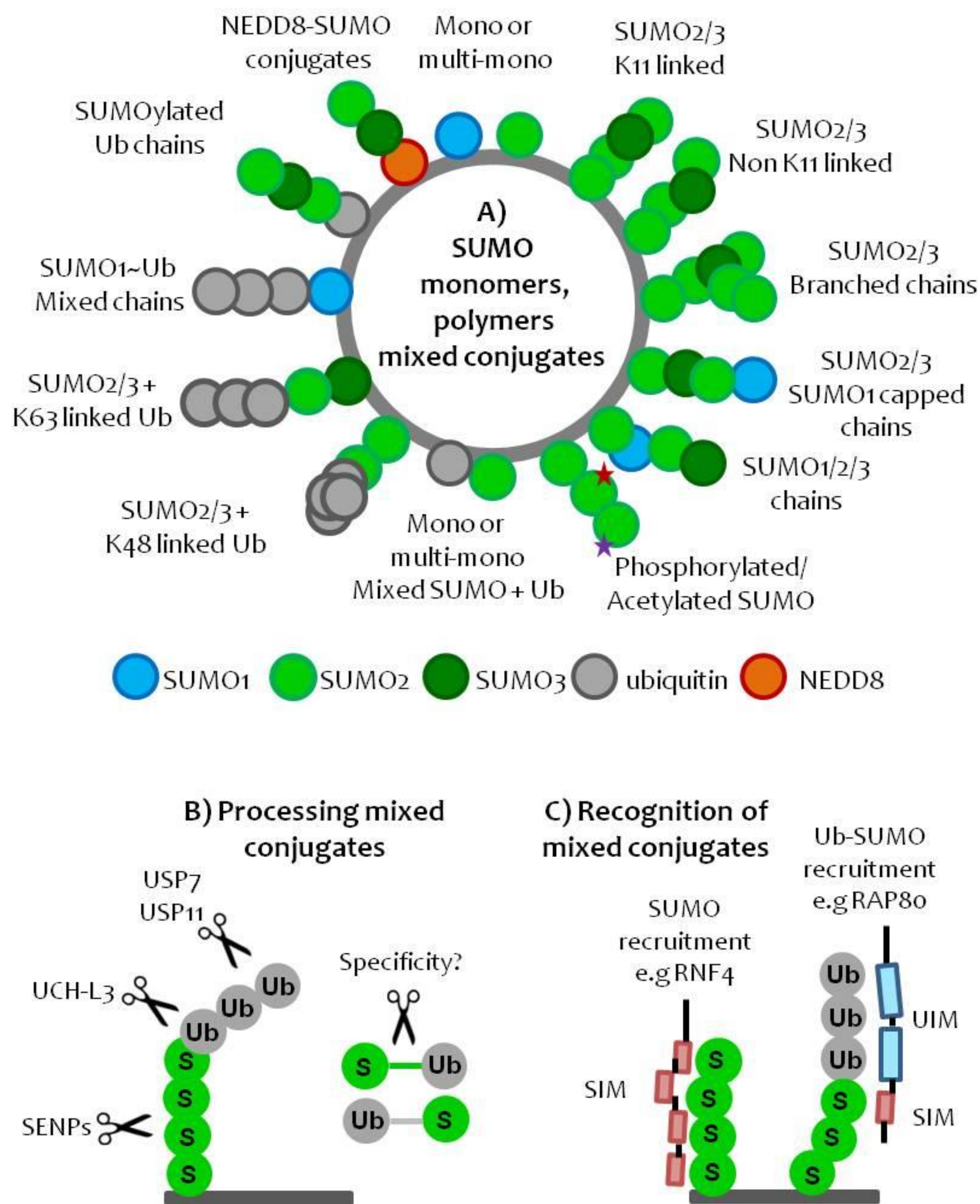


**Figure 2. Complexity of SUMO and SUMO~Ub conjugates**

A) SUMOylation, like ubiquitination occurs as mono, multimono and polymers but with the added complexity of multiple paralogs compared to a single ubiquitin. Mixed linkages of SUMO, ubiquitin and NEDD8 and modification by other PTMs such as phosphorylation and acetylation ("modified modifiers") add an additional layer of complexity. B) Mixed SUMO~Ub linkages require processing by DUBs and SENPs. If the presence of SUMO within Ub chains alters DUB activity or if Ub within SUMO chains affects SENP activity is unknown. Enzymes that specifically recognise mixed linkages have yet to be identified, but several DUBs have the ability to cleave ubiquitin chains from SUMO. C) Tandem SUMO and Ub recognition modules in proteins such as RAP80 "read" mixed linkages while tandem SUMO Interacting Motifs (SIMs) in proteins such as RNF4 recognise SUMO2/3 polymers.

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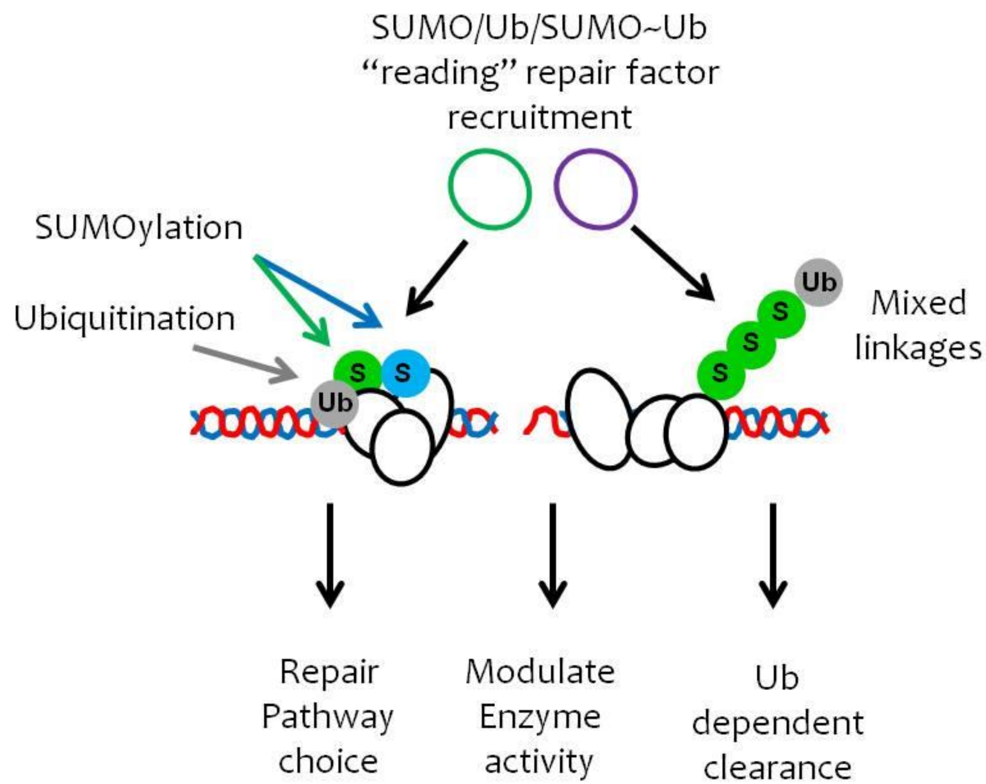


Graphical abstract

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Research highlights

- SUMO and ubiquitin have both distinct and overlapping functions in DSB repair.
- Mixed SUMO~Ub polymers impart additional layers of complexity and specificity to DSB repair such as the dual recognition of mixed conjugates by “reader” proteins such as RAP80.
- SUMOylation co-operates with ubiquitination through promoting recruitment and regulating the activity of ubiquitin ligases and de-ubiquitinating enzymes involved in DSB repair.

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