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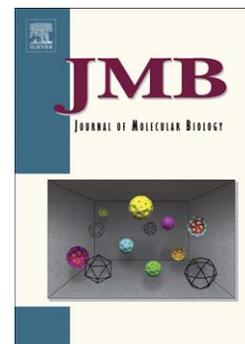
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SUMO in the DNA Double-Stranded Break Response: Similarities, Differences and Co-operation with Ubiquitin.

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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 “ ψ KxE/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

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 led to their collective designation as SUMO2/3. However some differences have been noted, SUMO3 contains a phosphorylation site (Ser²) 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 deSUMOylation

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 (SEN1-3 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

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

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

forks can promote the formation of DSBs. Their repair involves the localised recruitment of DNA repair sensors and effector 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 γ H2AX (H2AX phosphorylated at Ser¹³⁹), 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

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 *I SceI* 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.

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.

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

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].

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.

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References

1. Desterro, J.M., J. Thomson, and R.T. Hay, *Ubch9 conjugates SUMO but not ubiquitin*. FEBS Lett, 1997. **417**(3): p. 297-300.

2. Giraud, M.F., J.M.P. Desterro, and J.H. Naismith, *Structure of ubiquitin-conjugating enzyme 9 displays significant differences with other ubiquitin-conjugating enzymes which may reflect its specificity for sumo rather than ubiquitin*. *Acta Crystallographica Section D-Biological Crystallography*, 1998. **54**: p. 891-898.
3. Komander, D. and M. Rape, *The Ubiquitin Code*, in *Annual Review of Biochemistry*, Vol 81, R.D. Kornberg, Editor. 2012. p. 203-229.
4. Kamitani, T., et al., *Characterization of a second member of the sentrin family of ubiquitin-like proteins*. *Journal of Biological Chemistry*, 1998. **273**(18): p. 11349-11353.
5. Tatham, M.H., Y. Chen, and R.T. Hay, *Role of two residues proximal to the active site of Ubc9 in substrate recognition by the Ubc9 center dot SUMO-1 thiolester complex*. *Biochemistry*, 2003. **42**(11): p. 3168-3179.
6. Lamoliatte, F., et al., *Uncovering the SUMOylation and ubiquitylation crosstalk in human cells using sequential peptide immunopurification*. *Nat Commun*, 2017. **8**: p. 14109.
7. Hendriks, I.A., et al., *Site-specific mapping of the human SUMO proteome reveals co-modification with phosphorylation*. *Nat Struct Mol Biol*, 2017. **24**(3): p. 325-336.
8. Sampson, D.A., M. Wang, and M.J. Matunis, *The small ubiquitin-like modifier-1 (SUMO-1) consensus sequence mediates Ubc9 binding and is essential for SURIO-1 modification*. *Journal of Biological Chemistry*, 2001. **276**(24): p. 21664-21669.
9. Morris, J.R., et al., *The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress*. *Nature*, 2009. **462**(7275): p. 886-U77.
10. Galanty, Y., et al., *Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks*. *Nature*, 2009. **462**(7275): p. 935-U132.
11. Shima, H., et al., *Activation of the SUMO modification system is required for the accumulation of RAD51 at sites of DNA damage*. *Journal of Cell Science*, 2013. **126**(22): p. 5284-5292.
12. Streich, F.C. and C.D. Lima, *Structural and Functional Insights to Ubiquitin-Like Protein Conjugation*, in *Annual Review of Biophysics*, Vol 43, K.A. Dill, Editor. 2014. p. 357-379.
13. Shen, Z.Y., et al., *UBL1, a human ubiquitin-like protein associating with human RAD51/RAD52 proteins*. *Genomics*, 1996. **36**(2): p. 271-279.
14. Boddy, M.N., et al., *PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukaemia*. *Oncogene*, 1996. **13**(5): p. 971-982.
15. Matunis, M.J., E. Coutavas, and G. Blobel, *A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex*. *Journal of Cell Biology*, 1996. **135**(6): p. 1457-1470.
16. Mahajan, R., et al., *A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2*. *Cell*, 1997. **88**(1): p. 97-107.
17. Zhou, H.J., et al., *Toward a Comprehensive Characterization of a Human Cancer Cell Phosphoproteome*. *Journal of Proteome Research*, 2013. **12**(1): p. 260-271.
18. Matic, I., et al., *In vivo identification of human small ubiquitin-like modifier polymerization sites by high accuracy mass spectrometry and an in vitro to in vivo strategy*. *Molecular & Cellular Proteomics*, 2008. **7**(1): p. 132-144.
19. Xu, Z. and S.W.N. Au, *Mapping residues of SUMO precursors essential in differential maturation by SUMO-specific protease, SENP1*. *Biochemical Journal*, 2005. **386**: p. 325-330.
20. Reverter, D. and C.D. Lima, *A basis for SUMO protease specificity provided by analysis of human Senp2 and a Senp2-SUMO complex*. *Structure*, 2004. **12**(8): p. 1519-1531.
21. Sang, J., et al., *SUMO2 and SUMO3 transcription is differentially regulated by oxidative stress in an Sp1-dependent manner*. *Biochemical Journal*, 2011. **435**: p. 489-498.
22. Guo, Z.G., et al., *Sequential Posttranslational Modifications Program FEN1 Degradation during Cell-Cycle Progression*. *Molecular Cell*, 2012. **47**(3): p. 444-456.

23. Owerbach, D., et al., *A proline-90 residue unique to SUMO-4 prevents maturation and sumoylation*. Biochemical and Biophysical Research Communications, 2005. **337**(2): p. 517-520.
24. Wei, W.Z., et al., *A stress-dependent SUMO4 sumoylation of its substrate proteins*. Biochemical and Biophysical Research Communications, 2008. **375**(3): p. 454-459.
25. Liang, Y.C., et al., *SUMO5, a Novel Poly-SUMO Isoform, Regulates PML Nuclear Bodies*. Scientific Reports, 2016. **6**.
26. Su, H.L. and S.S.L. Li, *Molecular features of human ubiquitin-like SUMO genes and their encoded proteins*. Gene, 2002. **296**(1-2): p. 65-73.
27. Becker, J., et al., *Detecting endogenous SUMO targets in mammalian cells and tissues*. Nature Structural & Molecular Biology, 2013. **20**(4): p. 525-+.
28. Golebiowski, F., et al., *System-Wide Changes to SUMO Modifications in Response to Heat Shock*. Science Signaling, 2009. **2**(72).
29. Castoralova, M., et al., *SUMO-2/3 conjugates accumulating under heat shock or MG132 treatment result largely from new protein synthesis*. Biochimica Et Biophysica Acta-Molecular Cell Research, 2012. **1823**(4): p. 911-919.
30. Nayak, A. and S. Muller, *SUMO-specific proteases/isopeptidases: SENPs and beyond*. Genome Biology, 2014. **15**(7).
31. Shin, E.J., et al., *DeSUMOylating isopeptidase: a second class of SUMO protease*. Embo Reports, 2012. **13**(4): p. 339-346.
32. Schulz, S., et al., *Ubiquitin-specific protease-like 1 (USPL1) is a SUMO isopeptidase with essential, non-catalytic functions*. Embo Reports, 2012. **13**(10): p. 930-938.
33. Mendes, A.V., et al., *Evaluation of the activity and substrate specificity of the human SENP family of SUMO proteases*. Biochimica Et Biophysica Acta-Molecular Cell Research, 2016. **1863**(1): p. 139-147.
34. Sharma, P., et al., *Senp1 Is Essential for Desumoylating Sumo1-Modified Proteins but Dispensable for Sumo2 and Sumo3 Deconjugation in the Mouse Embryo*. Cell Reports, 2013. **3**(5): p. 1640-1650.
35. Pinto, M.P., et al., *Heat shock induces a massive but differential inactivation of SUMO-specific proteases*. Biochimica Et Biophysica Acta-Molecular Cell Research, 2012. **1823**(10): p. 1958-1966.
36. Shen, L.N., et al., *Characterization of SENP7, a SUMO-2/3-specific isopeptidase*. Biochemical Journal, 2009. **421**: p. 223-230.
37. Lima, C.D. and D. Reverter, *Structure of the Human SENP7 Catalytic Domain and Poly-SUMO Deconjugation Activities for SENP6 and SENP7*. Journal of Biological Chemistry, 2008. **283**(46): p. 32045-32055.
38. Kolli, N., et al., *Distribution and paralogue specificity of mammalian deSUMOylating enzymes*. Biochemical Journal, 2010. **430**: p. 335-344.
39. Tatham, M.H., et al., *RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation*. Nature Cell Biology, 2008. **10**(5): p. 538-546.
40. Sun, H., J.D. Levenson, and T. Hunter, *Conserved function of RNF4 family proteins in eukaryotes: targeting a ubiquitin ligase to SUMOylated proteins*. Embo Journal, 2007. **26**(18): p. 4102-4112.
41. Hang, J. and M. Dasso, *Association of the human SUMO-1 protease SENP2 with the nuclear pore*. Journal of Biological Chemistry, 2002. **277**(22): p. 19961-19966.
42. Gong, L. and E.T.H. Yeh, *Characterization of a family of nucleolar SUMO-specific proteases with preference for SUMO-2 or SUMO-3*. Journal of Biological Chemistry, 2006. **281**(23): p. 15869-15877.
43. Maison, C., et al., *The SUMO protease SENP7 is a critical component to ensure HP1 enrichment at pericentric heterochromatin*. Nature Structural & Molecular Biology, 2012. **19**(4): p. 458-460.

44. Bawa-Khalfe, T., et al., *Differential expression of SUMO-specific protease 7 variants regulates epithelial-mesenchymal transition*. Proceedings of the National Academy of Sciences of the United States of America, 2012. **109**(43): p. 17466-17471.
45. Garvin, A.J., et al., *The deSUMOylase SENP7 promotes chromatin relaxation for homologous recombination DNA repair*. Embo Reports, 2013. **14**(11): p. 975-983.
46. Evdokimov, E., et al., *Loss of SUMO1 in mice affects RanGAP1 localization and formation of PML nuclear bodies, but is not lethal as it can be compensated by SUMO2 or SUMO3*. Journal of Cell Science, 2008. **121**(24): p. 4106-4113.
47. Zhang, F.P., et al., *Sumo-1 function is dispensable in normal mouse development*. Molecular and Cellular Biology, 2008. **28**(17): p. 5381-5390.
48. Wang, L.L., et al., *SUMO2 is essential while SUMO3 is dispensable for mouse embryonic development*. Embo Reports, 2014. **15**(8): p. 878-885.
49. Tatham, M.H., et al., *Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9*. Journal of Biological Chemistry, 2001. **276**(38): p. 35368-35374.
50. Hendriks, I.A. and A.C.O. Vertegaal, *A comprehensive compilation of SUMO proteomics*. Nature Reviews Molecular Cell Biology, 2016. **17**(9): p. 581-595.
51. Tammsalu, T., et al., *Proteome-Wide Identification of SUMO2 Modification Sites*. Science Signaling, 2014. **7**(323).
52. Kulathu, Y. and D. Komander, *Atypical ubiquitylation - the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages*. Nature Reviews Molecular Cell Biology, 2012. **13**(8): p. 508-523.
53. Yau, R. and M. Rape, *The increasing complexity of the ubiquitin code*. Nature Cell Biology, 2016. **18**(6): p. 579-586.
54. Sun, H.Y. and T. Hunter, *Poly-Small Ubiquitin-like Modifier (PolySUMO)-binding Proteins Identified through a String Search*. Journal of Biological Chemistry, 2012. **287**(50): p. 42071-42083.
55. Searle, M.S., et al., *Structural insights into specificity and diversity in mechanisms of ubiquitin recognition by ubiquitin-binding domains*. Biochemical Society Transactions, 2012. **40**: p. 404-408.
56. Hecker, C.M., et al., *Specification of SUMO1- and SUMO2-interacting motifs*. Journal of Biological Chemistry, 2006. **281**(23): p. 16117-16127.
57. Sekiyama, N., et al., *Structure of the Small Ubiquitin-like Modifier (SUMO)-interacting Motif of MBD1-containing Chromatin-associated Factor 1 Bound to SUMO-3*. Journal of Biological Chemistry, 2008. **283**(51): p. 35966-35975.
58. Namanja, A.T., et al., *Insights into High Affinity Small Ubiquitin-like Modifier (SUMO) Recognition by SUMO-interacting Motifs (SIMs) Revealed by a Combination of NMR and Peptide Array Analysis*. Journal of Biological Chemistry, 2012. **287**(5): p. 3231-3240.
59. Stehmeier, P. and S. Muller, *Phospho-Regulated SUMO Interaction Modules Connect the SUMO System to CK2 Signaling*. Molecular Cell, 2009. **33**(3): p. 400-409.
60. Chang, C.C., et al., *Structural and Functional Roles of Daxx SIM Phosphorylation in SUMO Para log-Selective Binding and Apoptosis Modulation*. Molecular Cell, 2011. **42**(1): p. 62-74.
61. Tatham, M.H., et al., *Unique binding interactions among Ubc9, SUMO and RanBP2 reveal a mechanism for SUMO paralog selection*. Nature Structural & Molecular Biology, 2005. **12**(1): p. 67-74.
62. Gareau, J.R. and C.D. Lima, *The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition*. Nature Reviews Molecular Cell Biology, 2010. **11**(12): p. 861-871.
63. Meulmeester, E., et al., *Mechanism and consequences for paralog-specific sumoylation of ubiquitin-specific protease 25*. Molecular Cell, 2008. **30**(5): p. 610-619.

64. Eisenhardt, N., et al., *A new vertebrate SUMO enzyme family reveals insights into SUMO-chain assembly*. Nature Structural & Molecular Biology, 2015. **22**(12): p. 959-967.
65. Cappadocia, L., A. Pichler, and C.D. Lima, *Structural basis for catalytic activation by the human ZNF451 SUMO E3 ligase*. Nature Structural & Molecular Biology, 2015. **22**(12): p. 968-975.
66. Knipscheer, P., et al., *Noncovalent interaction between Ubc9 and SUMO promotes SUMO chain formation*. Embo Journal, 2007. **26**(11): p. 2797-2807.
67. Pilla, E., et al., *A Novel SUMO1-specific Interacting Motif in Dipeptidyl Peptidase 9 (DPP9) That Is Important for Enzymatic Regulation*. Journal of Biological Chemistry, 2012. **287**(53): p. 44320-44329.
68. Guzzo, C.M., et al., *Characterization of the SUMO-Binding Activity of the Myeloproliferative and Mental Retardation (MYM)-Type Zinc Fingers in ZNF261 and ZNF198*. Plos One, 2014. **9**(8).
69. Danielsen, J.R., et al., *DNA damage-inducible SUMOylation of HERC2 promotes RNF8 binding via a novel SUMO-binding Zinc finger*. Journal of Cell Biology, 2012. **197**(2): p. 179-187.
70. Diehl, C., et al., *Structural Analysis of a Complex between Small Ubiquitin-like Modifier 1 (SUMO1) and the ZZ Domain of CREB-binding Protein (CBP/p300) Reveals a New Interaction Surface on SUMO*. Journal of Biological Chemistry, 2016. **291**(24): p. 12658-+.
71. Leung, J.W., et al., *ZMYM3 regulates BRCA1 localization at damaged chromatin to promote DNA repair*. Genes Dev, 2017. **31**(3): p. 260-274.
72. Ciechanover, A., *The unravelling of the ubiquitin system*. Nature Reviews Molecular Cell Biology, 2015. **16**(5): p. 322-324.
73. Schimmel, J., et al., *The Ubiquitin-Proteasome System Is a Key Component of the SUMO-2/3 Cycle*. Molecular & Cellular Proteomics, 2008. **7**(11): p. 2107-2122.
74. Ceccaldi, R., B. Rondinelli, and A.D. D'Andrea, *Repair Pathway Choices and Consequences at the Double-Strand Break*. Trends Cell Biol, 2016. **26**(1): p. 52-64.
75. Yin, Y.L., et al., *SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage*. Genes & Development, 2012. **26**(11): p. 1196-1208.
76. Hendriks, I.A., et al., *SUMO-2 Orchestrates Chromatin Modifiers in Response to DNA Damage*. Cell Reports, 2015. **10**(10): p. 1778-1791.
77. Galanty, Y., et al., *RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair*. Genes & Development, 2012. **26**(11): p. 1179-1195.
78. Vyas, R., et al., *RNF4 is required for DNA double-strand break repair in vivo*. Cell Death and Differentiation, 2013. **20**(3): p. 490-502.
79. Pfeiffer, A., et al., *Ataxin-3 consolidates the MDC1-dependent DNA double-strand break response by counteracting the SUMO-targeted ubiquitin ligase RNF4*. EMBO J, 2017.
80. Hu, X., A. Paul, and B. Wang, *Rap80 Protein Recruitment to DNA Double-strand Breaks Requires Binding to Both Small Ubiquitin-like Modifier (SUMO) and Ubiquitin Conjugates*. Journal of Biological Chemistry, 2012. **287**(30): p. 25510-25519.
81. Ismail, I.H., et al., *CBX4-mediated SUMO modification regulates BMI1 recruitment at sites of DNA damage*. Nucleic Acids Research, 2012. **40**(12): p. 5497-5510.
82. Luo, K.T., et al., *Sumoylation of MDC1 is important for proper DNA damage response*. Embo Journal, 2012. **31**(13): p. 3008-3019.
83. Chen, W.T., et al., *Systematic Identification of Functional Residues in Mammalian Histone H2AX*. Molecular and Cellular Biology, 2013. **33**(1): p. 111-126.
84. Bologna, S., et al., *Sumoylation regulates EXO1 stability and processing of DNA damage*. Cell Cycle, 2015. **14**(15): p. 2439-2450.
85. Hu, Y.H. and J.D. Parvin, *Small Ubiquitin-like Modifier (SUMO) Isoforms and Conjugation-independent Function in DNA Double-strand Break Repair Pathways*. Journal of Biological Chemistry, 2014. **289**(31): p. 21289-21295.

86. Morris, J.R. and E. Solomon, *BRCA1 : BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair*. Human Molecular Genetics, 2004. **13**(8): p. 807-817.
87. Newton, K., et al., *Ubiquitin chain editing revealed by polyubiquitin linkage-specific antibodies*. Cell, 2008. **134**(4): p. 668-678.
88. van Wijk, S.J.L., et al., *Fluorescence-Based Sensors to Monitor Localization and Functions of Linear and K63-Linked Ubiquitin Chains in Cells*. Molecular Cell, 2012. **47**(5): p. 797-809.
89. Thorslund, T., et al., *Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage*. Nature, 2015. **527**(7578): p. 389-+.
90. Dou, H., et al., *Regulation of DNA Repair through DeSUMOylation and SUMOylation of Replication Protein A Complex*. Molecular Cell, 2010. **39**(3): p. 333-345.
91. Aguilar-Martinez, E., et al., *Screen for multi-SUMO-binding proteins reveals a multi-SIM-binding mechanism for recruitment of the transcriptional regulator ZMYM2 to chromatin*. Proceedings of the National Academy of Sciences of the United States of America, 2015. **112**(35): p. E4854-E4863.
92. Nakada, S., et al., *Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1*. Nature, 2010. **466**(7309): p. 941-U59.
93. Butler, L.R., et al., *The proteasomal de-ubiquitinating enzyme POH1 promotes the double-strand DNA break response*. Embo Journal, 2012. **31**(19): p. 3918-3934.
94. Sharma, N., et al., *USP3 counteracts RNF168 via deubiquitinating H2A and gamma H2AX at lysine 13 and 15*. Cell Cycle, 2014. **13**(1): p. 106-114.
95. Nishi, R., et al., *Systematic characterization of deubiquitylating enzymes for roles in maintaining genome integrity*. Nature Cell Biology, 2014. **16**(10): p. 1016-1026.
96. Kee, Y. and T.T. Huang, *Role of Deubiquitinating Enzymes in DNA Repair*. Molecular and Cellular Biology, 2016. **36**(4): p. 524-544.
97. Goodarzi, A.A., T. Kurka, and P.A. Jeggo, *KAP-1 phosphorylation regulates CHD3 nucleosome remodeling during the DNA double-strand break response*. Nature Structural & Molecular Biology, 2011. **18**(7): p. 831-U112.
98. Hendriks, I.A., et al., *Ubiquitin-specific Protease 11 (USP11) Deubiquitinates Hybrid Small Ubiquitin-like Modifier (SUMO)-Ubiquitin Chains to Counteract RING Finger Protein 4 (RNF4)*. Journal of Biological Chemistry, 2015. **290**(25): p. 15526-15537.
99. Lecona, E., et al., *USP7 is a SUMO deubiquitinase essential for DNA replication*. Nature Structural & Molecular Biology, 2016. **23**(4): p. 270-277.
100. Zhu, Q.Z., et al., *USP7 deubiquitinase promotes ubiquitin-dependent DNA damage signaling by stabilizing RNF168**. Cell Cycle, 2015. **14**(9): p. 1413-1425.
101. Zlatanou, A., et al., *USP7 is essential for maintaining Rad18 stability and DNA damage tolerance*. Oncogene, 2016. **35**(8): p. 965-976.
102. Schoenfeld, A.R., et al., *BRCA2 is ubiquitinated in vivo and interacts with USP11, a deubiquitinating enzyme that exhibits prosurvival function in the cellular response to DNA damage*. Molecular and Cellular Biology, 2004. **24**(17): p. 7444-7455.
103. Wiltshire, T.D., et al., *Sensitivity to Poly(ADP-ribose) Polymerase (PARP) Inhibition Identifies Ubiquitin-specific Peptidase 11 (USP11) as a Regulator of DNA Double-strand Break Repair*. Journal of Biological Chemistry, 2010. **285**(19): p. 14565-14571.
104. Orthwein, A., et al., *A mechanism for the suppression of homologous recombination in G1 cells*. Nature, 2015. **528**(7582): p. 422-+.
105. Bett, J.S., et al., *Ubiquitin C-terminal hydrolases cleave isopeptide- and peptide-linked ubiquitin from structured proteins but do not edit ubiquitin homopolymers*. Biochemical Journal, 2015. **466**: p. 489-498.
106. Luo, K.T., et al., *A phosphorylation-deubiquitination cascade regulates the BRCA2-RAD51 axis in homologous recombination*. Genes & Development, 2016. **30**(23): p. 2581-2595.

107. Brinkmann, K., et al., *Regulation of the DNA damage response by ubiquitin conjugation*. *Frontiers in Genetics*, 2015. **6**.
108. Citterio, E., *Fine-tuning the ubiquitin code at DNA double-strand breaks: deubiquitinating enzymes at work*. *Frontiers in Genetics*, 2015. **6**.
109. Schwertman, P., S. Bekker-Jensen, and N. Mailand, *Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers*. *Nature Reviews Molecular Cell Biology*, 2016. **17**(6): p. 379-394.
110. Bekker-Jensen, S., et al., *HERC2 coordinates ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes*. *Nature Cell Biology*, 2010. **12**(1): p. 80-U209.
111. Shire, K., et al., *Identification of RNF168 as a PML nuclear body regulator*. *Journal of Cell Science*, 2016. **129**(3): p. 580-591.
112. Fradet-Turcotte, A., et al., *53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark*. *Nature*, 2013. **499**(7456): p. 50-+.
113. Mattioli, F., et al., *RNF168 Ubiquitinates K13-15 on H2A/H2AX to Drive DNA Damage Signaling*. *Cell*, 2012. **150**(6): p. 1182-1195.
114. Wang, B., et al., *Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response*. *Science*, 2007. **316**(5828): p. 1194-1198.
115. Sobhian, B., et al., *RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites*. *Science*, 2007. **316**(5828): p. 1198-1202.
116. Kim, H., J.J. Chen, and X.H. Yu, *Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response*. *Science*, 2007. **316**(5828): p. 1202-1205.
117. Yan, J., et al., *The ubiquitin-interacting motif-containing protein RAP80 interacts with BRCA1 and functions in DNA damage repair response*. *Cancer Research*, 2007. **67**(14): p. 6647-6656.
118. Guzzo, C.M., et al., *DNA DAMAGE RNF4-Dependent Hybrid SUMO-Ubiquitin Chains Are Signals for RAP80 and Thereby Mediate the Recruitment of BRCA1 to Sites of DNA Damage*. *Science Signaling*, 2012. **5**(253).
119. Anamika and L. Spyropoulos, *Molecular Basis for Phosphorylation-dependent SUMO Recognition by the DNA Repair Protein RAP80*. *Journal of Biological Chemistry*, 2016. **291**(9): p. 4417-4428.
120. Branigan, E., et al., *Structural basis for the RING-catalyzed synthesis of K63-linked ubiquitin chains*. *Nature Structural & Molecular Biology*, 2015. **22**(8): p. 597-602.
121. Rojas-Fernandez, A., et al., *SUMO Chain-Induced Dimerization Activates RNF4*. *Molecular Cell*, 2014. **53**(6): p. 880-892.
122. Kalb, R., et al., *BRCA1 Is a Histone-H2A-Specific Ubiquitin Ligase*. *Cell Reports*, 2014. **8**(4): p. 999-1005.
123. Densham, R.M., et al., *Human BRCA1-BARD1 ubiquitin ligase activity counteracts chromatin barriers to DNA resection*. *Nature Structural & Molecular Biology*, 2016. **23**(7): p. 647-655.
124. Ismail, I.H., et al., *BMI1-mediated histone ubiquitylation promotes DNA double-strand break repair*. *Journal of Cell Biology*, 2010. **191**(1): p. 45-60.
125. Leung, J.W., et al., *Nucleosome Acidic Patch Promotes RNF168-and RING1B/BMI1-Dependent H2AX and H2A Ubiquitination and DNA Damage Signaling*. *Plos Genetics*, 2014. **10**(3).
126. Ginja, V., et al., *BMI1 Is Recruited to DNA Breaks and Contributes to DNA Damage-Induced H2A Ubiquitination and Repair*. *Molecular and Cellular Biology*, 2011. **31**(10): p. 1972-1982.
127. Sahtoe, D.D. and T.K. Sixma, *Layers of DUB regulation*. *Trends in Biochemical Sciences*, 2015. **40**(8): p. 456-467.
128. Zhen, Y., et al., *Regulation of USP28 Deubiquitinating Activity by SUMO Conjugation*. *Journal of Biological Chemistry*, 2014. **289**(50): p. 34838-34850.
129. Knobel, P.A., et al., *USP28 Is Recruited to Sites of DNA Damage by the Tandem BRCT Domains of 53BP1 but Plays a Minor Role in Double-Strand Break Metabolism*. *Molecular and Cellular Biology*, 2014. **34**(11): p. 2062-2074.

130. Cuella-Martin, R., et al., *53BP1 Integrates DNA Repair and p53-Dependent Cell Fate Decisions via Distinct Mechanisms*. *Molecular Cell*, 2016. **64**(1): p. 51-64.
131. Guervilly, J.H. and P.H.L. Gaillard, *SLX4 gains weight with SUMO in genome maintenance*. *Molecular & Cellular Oncology*, 2016. **3**(2).
132. Guervilly, J.H., et al., *The SLX4 Complex Is a SUMO E3 Ligase that Impacts on Replication Stress Outcome and Genome Stability*. *Molecular Cell*, 2015. **57**(1): p. 123-137.
133. Ouyang, J., et al., *Noncovalent Interactions with SUMO and Ubiquitin Orchestrate Distinct Functions of the SLX4 Complex in Genome Maintenance*. *Molecular Cell*, 2015. **57**(1): p. 108-122.
134. Lachaud, C., et al., *Distinct functional roles for the two SLX4 ubiquitin-binding UBZ domains mutated in Fanconi anemia*. *Journal of Cell Science*, 2014. **127**(13): p. 2811-2817.
135. Gonzalez-Prieto, R., et al., *SUMOylation and PARylation cooperate to recruit and stabilize SLX4 at DNA damage sites*. *Embo Reports*, 2015. **16**(4): p. 512-519.
136. Eladad, S., et al., *Intra-nuclear trafficking of the BLM helicase to DNA damage-induced foci is regulated by SUMO modification*. *Human Molecular Genetics*, 2005. **14**(10): p. 1351-1365.
137. Psakhye, I. and S. Jentsch, *Protein Group Modification and Synergy in the SUMO Pathway as Exemplified in DNA Repair*. *Cell*, 2012. **151**(4): p. 807-820.
138. Wu, C.S., et al., *SUMOylation of ATRIP potentiates DNA damage signaling by boosting multiple protein interactions in the ATR pathway*. *Genes & Development*, 2014. **28**(13): p. 1472-1484.
139. Bohm, S., et al., *Disruption of SUMO-targeted ubiquitin ligases Slx5-Slx8/RNF4 alters RecQ-like helicase Sgs1/BLM localization in yeast and human cells*. *DNA Repair*, 2015. **26**: p. 1-14.
140. Kuo, C.Y., et al., *An Arginine-rich Motif of Ring Finger Protein 4 (RNF4) Oversees the Recruitment and Degradation of the Phosphorylated and SUMOylated Kruppel-associated Box Domain-associated Protein 1 (KAP1)/TRIM28 Protein during Genotoxic Stress*. *Journal of Biological Chemistry*, 2014. **289**(30): p. 20757-20772.
141. Franz, A., L. Ackermann, and T. Hoppe, *Ring of Change: CDC48/p97 Drives Protein Dynamics at Chromatin*. *Frontiers in Genetics*, 2016. **7**.
142. Dantuma, N.P., K. Acs, and M.S. Luijsterburg, *Should I stay or should I go: VCP/p97-mediated chromatin extraction in the DNA damage response*. *Experimental Cell Research*, 2014. **329**(1): p. 9-17.
143. Meyer, H. and C.C. Wehl, *The VCP/p97 system at a glance: connecting cellular function to disease pathogenesis*. *Journal of Cell Science*, 2014. **127**(18): p. 3877-3883.
144. Acs, K., et al., *The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks*. *Nature Structural & Molecular Biology*, 2011. **18**(12): p. 1345-U55.
145. Meerang, M., et al., *The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks*. *Nature Cell Biology*, 2011. **13**(11): p. 1376-U204.
146. Nie, M.H., et al., *Dual Recruitment of Cdc48 (p97)-Ufd1-Npl4 Ubiquitin-selective Segregase by Small Ubiquitin-like Modifier Protein (SUMO) and Ubiquitin in SUMO-targeted Ubiquitin Ligase-mediated Genome Stability Functions*. *Journal of Biological Chemistry*, 2012. **287**(35): p. 29610-29619.
147. Bergink, S., et al., *Role of Cdc48/p97 as a SUMO-targeted segregase curbing Rad51-Rad52 interaction*. *Nature Cell Biology*, 2013. **15**(5): p. 526-+.
148. Kohler, J.B., et al., *Targeting of SUMO substrates to a Cdc48-Ufd1-Npl4 segregase and STUbL pathway in fission yeast*. *Nature Communications*, 2015. **6**.
149. Su, V. and A.F. Lau, *Ubiquitin-like and ubiquitin-associated domain proteins: significance in proteasomal degradation*. *Cellular and Molecular Life Sciences*, 2009. **66**(17): p. 2819-2833.
150. Boyd, L.K., et al., *Characterisation of the SUMO-Like Domains of Schizosaccharomyces pombe Rad60*. *Plos One*, 2010. **5**(9).

151. Prudden, J., et al., *DNA Repair and Global Sumoylation Are Regulated by Distinct Ubc9 Noncovalent Complexes*. *Molecular and Cellular Biology*, 2011. **31**(11): p. 2299-2310.
152. Yang, K.L., et al., *Regulation of the Fanconi anemia pathway by a SUMO-like delivery network*. *Genes & Development*, 2011. **25**(17): p. 1847-1858.
153. Liang, F.S., et al., *Promotion of RAD51-Mediated Homologous DNA Pairing by the RAD51AP1-UAF1 Complex*. *Cell Reports*, 2016. **15**(10): p. 2118-2126.
154. Cheema, A., et al., *Functional mimicry of the acetylated C-terminal tail of p53 by a SUMO-1 acetylated domain, SAD*. *J Cell Physiol*, 2010. **225**(2): p. 371-84.
155. Ullmann, R., et al., *An Acetylation Switch Regulates SUMO-Dependent Protein Interaction Networks*. *Molecular Cell*, 2012. **46**(6): p. 759-770.
156. Choudhary, C., et al., *The growing landscape of lysine acetylation links metabolism and cell signalling*. *Nature Reviews Molecular Cell Biology*, 2014. **15**(8): p. 536-550.
157. Wagner, S.A., et al., *A Proteome-wide, Quantitative Survey of In Vivo Ubiquitylation Sites Reveals Widespread Regulatory Roles*. *Molecular & Cellular Proteomics*, 2011. **10**(10).
158. Poulsen, S.L., et al., *RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response*. *Journal of Cell Biology*, 2013. **201**(6): p. 797-807.
159. van Cuijk, L., et al., *SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair*. *Nature Communications*, 2015. **6**.
160. Ma, T., et al., *RNF111-Dependent Neddylation Activates DNA Damage-Induced Ubiquitination*. *Molecular Cell*, 2013. **49**(5): p. 897-907.
161. Awasthi, P., M. Foiani, and A. Kumar, *ATM and ATR signaling at a glance*. *J Cell Sci*, 2015. **128**(23): p. 4255-62.
162. Huguenin-Dezot, N., et al., *Synthesis of Isomeric Phosphoubiquitin Chains Reveals that Phosphorylation Controls Deubiquitinase Activity and Specificity*. *Cell Reports*, 2016. **16**(4): p. 1180-1193.
163. Kazlauskaitė, A., et al., *Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser(65)*. *Biochemical Journal*, 2014. **460**: p. 127-139.
164. Wauer, T., et al., *Ubiquitin Ser65 phosphorylation affects ubiquitin structure, chain assembly and hydrolysis*. *Embo Journal*, 2015. **34**(3): p. 307-325.

SUMO paralogs and specificity

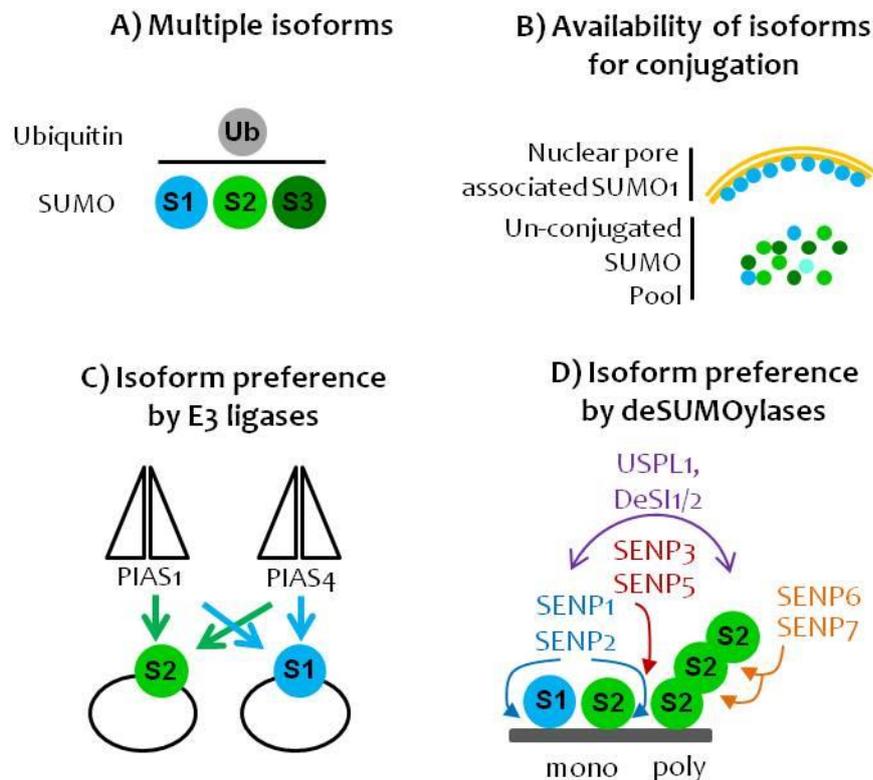


Figure 1. Paralog specific SUMOylation.

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.

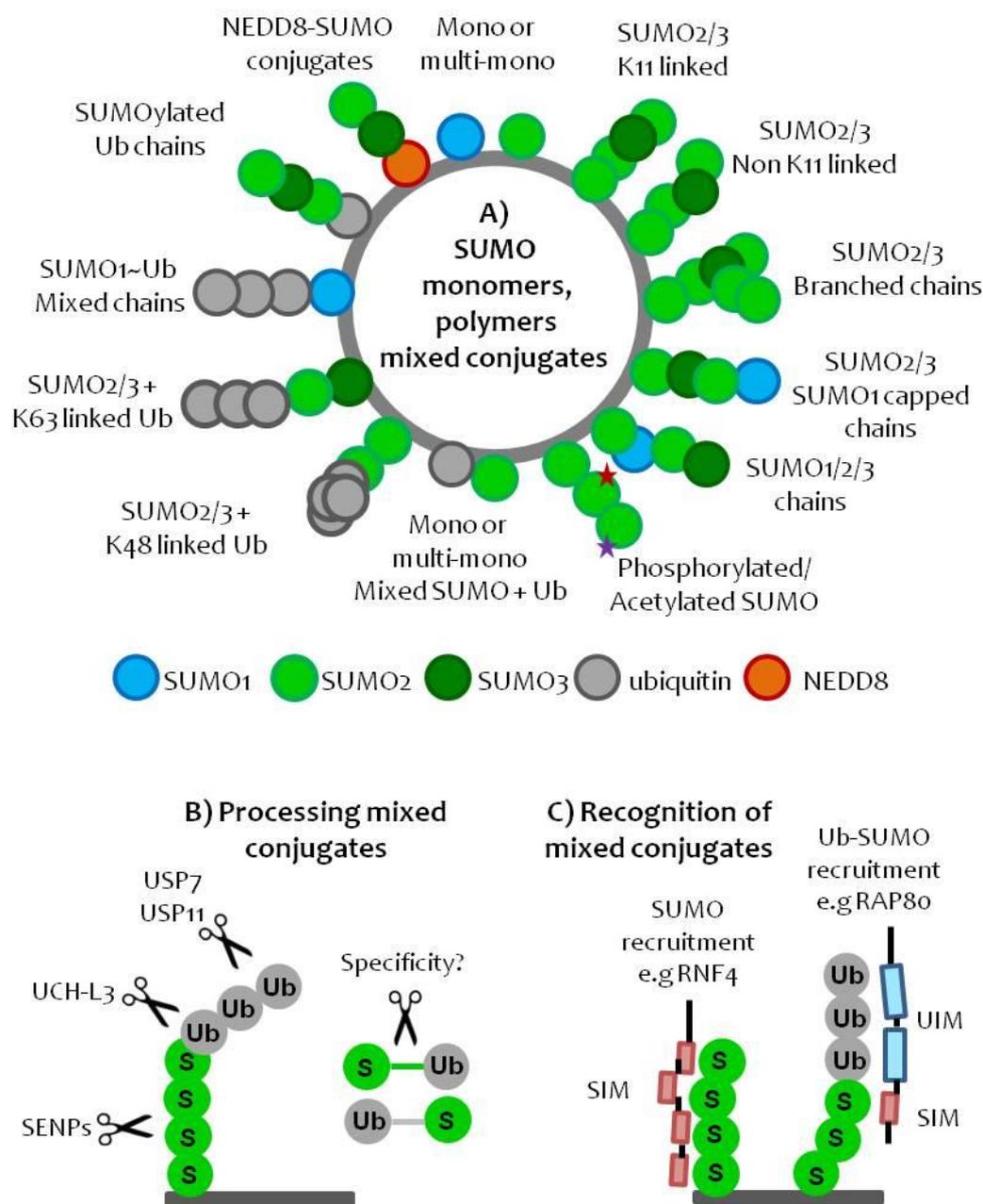
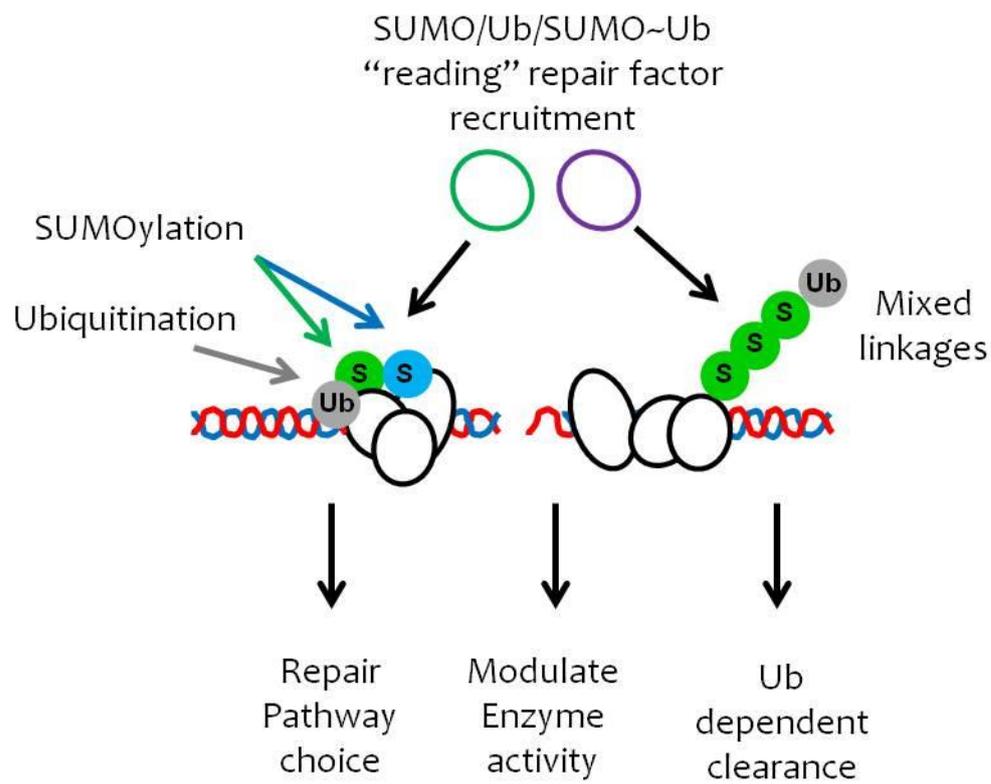


Figure 2. Complexity of SUMO and SUMO~Ub conjugates

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 recruitment modules in proteins such as RAP80 “read” mixed linkages while tandem SUMO Interacting Motifs (SIMs) in proteins such as RNF4 recognise SUMO2/3 polymers.



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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