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REVIEW

SUMO in the regulation of DNA repair and transcription at nuclear pores

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Two related post-translational modifications, the covalent linkage of Ubiquitin and the Small Ubiquitin-related MOdifier (SUMO) to lysine residues, play key roles in the regulation of both DNA repair pathway choice and transcription. Whereas ubiquitination is generally associated with proteasome-mediated protein degradation, the impact of sumoylation has been more mysterious. In the cell nucleus, sumoylation effects are largely mediated by the relocalization of the modified targets, particularly in response to DNA damage. This is governed in part by the concentration of SUMO protease at nuclear pores [Melchior, F *et al.* (2003) Trends Biochem Sci 28, 612–618; Ptak, C and Wozniak, RW (2017) Adv Exp Med Biol 963, 111–126]. We review here the roles of sumoylation in determining genomic locus positioning relative to the nuclear envelope and to nuclear pores, to facilitate repair and regulate transcription.

Keywords: damage relocation; DNA-protein crosslink; double-strand break repair; proteolysis; replication fork collapse; STUbL; SUMOylation; telomeres; transcription

Mechanisms of ubiquitination and sumoylation

Ubiquitination entails the covalent attachment of a small protein of 76 amino acid residues through its C-terminal glycine to a lysine in a target protein. It was originally discovered as a means to target modified substrates to the proteasome machinery, and thereby to regulate protein turnover [1]. Since then, many studies have shown that ubiquitination can also regulate protein activity without triggering degradation of its substrate [2]. Ubiquitin (Ub) moieties are conjugated either as a monomer or as a polymeric chain, which results from repetitive Ub addition. Distinct types of linkages within Ub chains trigger different outcomes; for example, Lys48-linked Ub chains target the modified protein to the proteasome, while Lys63-linked chains tend to regulate protein–protein interactions [2].

SUMO is a small Ub-like protein that becomes covalently attached to its substrate through a highly conserved cascade of enzymatic reactions mediated by E1, E2, and E3 ligases, like ubiquitination. Sumoylation has

Abbreviations
BIR, break-induced replication; CPT, camptothecin; DPC, DNA-protein crosslink; DSB, double-strand break; DUB, deubiquitinating complex; FRT, flippase recognition target; HP1, heterochromatin protein 1; HR, homologous recombination; HU, hydroxyurea; LADs, lamin-associated domains; MCM, mini-chromosome maintenance; MMEJ, microhomology-mediated end-joining; MRX, complex of yeast Mre11, Rad50 and Xrn2 (or MRN with NBS1 in man); NHEJ, non-homologous end joining; NPC, nuclear pore complex; PCNA, proliferating cell nuclear antigen; PML nuclear bodies, promyelocytic leukemia nuclear bodies; RFB, replication fork barrier; RPA, replication protein A; SCR, sister-chromatid recombination; SIM, SUMO-interacting motif; SIR, silent information regulator; SMCs, structural maintenance of chromosomes; STUbLs, SUMO-targeted E3 ubiquitin ligases; SUMO, Small Ubiquitin-related MOdifier; Top1cc, topoisomerase 1 covalent complex; Ub, Ubiquitin.

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been linked genetically to the regulation of various cellular processes, most notably transcription and the repair of DNA damage, and in many cases, it serves to ensure a specific subnuclear localization of the relevant DNA-dependent event [3–5].

Vertebrates have three SUMO proteins: SUMO-1 and the two highly related polypeptides, SUMO-2 and SUMO-3 (also called SUMO2/3), that function redundantly. Budding yeast has a single SUMO protein called Smt3 [6,7]. The enzymatic cascade that attaches SUMO to a target protein involves a heterodimeric activating enzyme E1 (Uba2/Aos1 in budding yeast) that transfers SUMO to the SUMO-conjugating enzyme E2 (Ubc9 in yeast), and the transfer of the SUMO residue to the target protein by an E3 ligase (budding yeast has four SUMO E3s: Siz1, Siz2, Zip3, and Mms21). In mammals there are a large number of Ub conjugating E1, E2, and E3 enzymes (e.g., there are more than 500 distinct E3 enzymes alone), while the SUMO-conjugation system is simpler, with one heterodimeric E1 activating enzyme (SAE1/SAE2 in mammals), a unique E2 ligase (UBC9) and a small number of E3 ligases with different, but sometimes overlapping, target specificities [8,9].

Like Ub, SUMO can be conjugated either as a monomer or as a polymeric chain. Mono-sumoylation mainly regulates protein–protein interactions through SUMO-interacting motifs (SIMs), which help assemble multisubunit complexes and mediate protein accumulation in subnuclear compartments such as NPCs or PML nuclear bodies [10,11]. In mammals, poly-SUMO chains are formed by SUMO2/3 linked through their Lys11 residue, while SUMO1 can act as a chain terminator, capping a poly-SUMO2/3 chain [6,7,12]. Poly-SUMO2/3 conjugates have been shown to play a critical role in the cellular response to stress, most notably DNA damage (reviewed in [13–15]).

Sumoylation is able to cross-talk directly with the Ub-proteasome system, through a set of SUMO-targeted E3 ubiquitin ligases (STUbLs), which can bind sumoylated lysines and target the poly-sumoylated substrate for poly-ubiquitination. There are two STUbLs in yeast, Uls1 and the Slx5/Slx8 complex (also called Uls2), and three in mammalian cells (RNF111, RNF4, and SHPRH, or SNF2 Histone Linker PHD RING Helicase, which has extensive homology to yeast Uls1). In addition, the *S. cerevisiae* repair factor Rad18 has a SIM that enhances PCNA ubiquitination by Rad18 *in vitro* [16,17]. STUbLs bind sumoylated proteins through one or multiple SIMs and catalyze ubiquitination, which usually leads to degradation of the substrate by the proteasome. Not only are many DNA repair factors subject to sumoylation [15,18], but STUbLs, as well as SUMO ligases, have been repeatedly implicated in DNA repair in yeast by genome-wide synthetic lethality or synthetic sensitivity screens [19–21].

Chromosome positioning during DNA repair

DNA repair in heterochromatin

Both the positioning of DNA damage within the nucleus, and the relative accessibility of the chromatin in which the damage is found influence the choice and efficacy of DNA repair mechanisms, in yeast [22] and in higher eukaryotes (reviewed in [23]). Indeed, several studies have demonstrated that double-strand breaks (DSBs) occurring in repetitive or heterochromatic domains, such as ribosomal DNA (rDNA) or pericentric heterochromatin, shift away from these domains to be repaired by homologous recombination (HR). This relocation is thought to minimize the risk of recombination-mediated rearrangements among repeats, which can result in sequence loss or chromosomal translocations [24]. It has also been argued, based on relative mutation rates, that DSBs in active genes are more often repaired by HR to prevent mutations in coding sequences, while DSBs in heterochromatin and lamin-associated domains (LADs) rely on more error-prone pathways, such as microhomology-mediated end-joining (MMEJ) or non-homologous end-joining (NHEJ) [25,26]. We note, however, that this bias may not hold in *Drosophila* [27].

Recent work has focused on how damage moves or shifts position, especially for DSBs that occur in the repetitive heterochromatin of pericentric and centromeric domains of flies and mammalian chromosomes. By tracking γH2Av damage foci in *Drosophila*, it was shown that irradiation-induced damage shifts away from heterochromatic domains to the nuclear periphery, presumably as a pre-requisite for repair by HR [28,29]. In mouse cells, cell-cycle-dependent differences were observed: in G1 phase, DSBs in pericentric heterochromatin were repaired by NHEJ and did not shift position, whereas in S or G2 phases, heterochromatic DSBs shifted to the periphery of the heterochromatin domain to enable HR [30]. Long before these discoveries, DSBs in the rDNA of budding yeast were shown to shift away from the nucleolus prior to forming Rad52 foci, which precede Rad51 loading for HR-mediated repair [31]. This relocation required the Smc5/Smc6 complex and mono-sumoylation by its associated SUMO ligase, Mms21 [31]. A similar SMC5/SMC6/MMS21-dependent shift of breaks out of the nucleolus was also confirmed in mammalian cells [32,33]. Sumoylation was thus shown to play a role in both damage relocation and in the regulation of repair.

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Mechanistically, it was proposed that mono-sumoylation mediated by MMS21/NSE2 (called dPIAS in flies) first generates a block-to-end resection and Rad51 loading, and then by attracting the STUbL/RENi complex (Slx5/Slx8 in yeast, Dgrn/dRad60 in flies and RNF4 in man) contributes to the stable relocalization of the damage to either the inner nuclear envelope or to NPCs [19,28,29,34,35]. The action of the STUbL, which ubiquitinates the sumoylated factors and tags them for degradation, appears to precede strand invasion for HR or break-induced replication (BIR, see Fig. 1A,B). Exactly which proteins are degraded and which repair components then bind the sites of damage, is not entirely resolved for persistent DSBs, although in the case of damage in the yeast rDNA the key target appeared to be Rad52, the functional homologue of BRCA2 in yeast [31]. The relocation of damage from the rDNA compartment (i.e., the nucleolus) may require the action of the p97/Cdc48 ATPase, which recognizes ubiquitinated and sumoylated substrates, and catalyzes either Ub unfolding or factor release [39]. Besides Rad52, another p97/Cdc48 target relevant for yeast rDNA DSB movement may be the phosphorylated and sumoylated form of CLIP-cohibin, a specialized complex containing components of the inner nuclear envelope and homodimers of Lrs4 and Csm1. This complex tethers the rDNA to the yeast's nuclear membrane in the absence of DNA damage [40].

The shared logic of these DSB relocation events across various species appears to be the reduction of illegitimate recombination or chromosomal translocations that occur when breaks arise in repetitive sequence. This is achieved by inhibiting HR, translocating the break, and finally releasing the inhibition through sequential sumoylation, ubiquitination, and repair factor degradation near the nuclear periphery (Fig. 1). The removal of one or more ligands that block the DNA end from appropriate processing, leads to alternative modes of repair than standard homology-driven recombination with the sister chromatid. Intriguingly, an inner nuclear membrane-associated endonuclease has recently been identified in humans that generates a 5′ overhang to favor repair by NHEJ, counteracting the resection that forms the 3′ overhang necessary for Rad51-mediated strand invasion [41]. This suggests that NHEJ may also be an alternative outcome for hard-to-repair breaks at the nuclear envelope.

Functional outcome—Why the nuclear periphery?

Several studies in budding yeast have examined the importance of relocating persistent DSBs generated in euchromatin to the NPC [19,42]. Horigome et al. showed that DSB relocation to the NPC indeed enabled or favored alternative pathways such as MMEJ, or BIR, while the association of resected breaks with a second perinuclear site, the SUN domain homolog Mps3, appeared to repress ectopic recombination at least transiently [43–45]. The shift of persistent DSBs to the yeast nuclear periphery occurred 1–2 h after endonuclease-induced DSB formation, and was triggered as well by collapsed replication forks in non-repetitive sequences, but not by simple fork stalling on hydroxyurea (HU) [19]. Relocation to the NPC in yeast required the Nup84 subcomplex and its interaction with the conserved SUMO-dependent E3 ligase Slx5/Slx8 [19,44], as described above for the shift of DSB damage out of heterochromatin or out of the nucleolus.

In both budding and fission yeast, a second inner nuclear envelope site for DSB accumulation was identified as the SUN domain protein, Mps3 or Sad1 [43,46]. In contrast to relocation to pores, the association of damage with Mps3 in budding yeast depended on DNA end resection, and on the INO80C chromatin remodeler, perhaps due to the association of INO80 with Cdc48 [40,45,46]. Resected DSBs without sister chromatid donors could bind Mps3 and be repaired by homology-based repair in S/G2 phase after a delay [5,43,44]. This relocation event required only mono-sumoylation of key substrates, such as RPA, Rad52, or Rad59, all of which play key roles in mitotic recombination [47,48]. In fission yeast, DSB relocation to the nuclear periphery in S/G2 phase led to repair focus formation at the *S. pombe* Mps3 homolog, Sad1, and promoted ectopic gene conversion [46,49]. It is possible that breaks associate first with SUN-domain proteins and then shift to the NPC or other sites for further processing [45], so that the two processing sites would contribute to repair sequentially, rather than being mutually exclusive.

Because repeat-containing telomeres cluster at the nuclear envelope in yeast [50], it was examined whether telomeric exchange by HR was also enhanced by their spatial clustering near nuclear pores. The Kupiec and Haber laboratories have demonstrated that genomic regions that are spatially juxtaposed do recombine more efficiently than sequences that are spatially distant from each other in yeast [51,52]. Their work reinforced earlier evidence that the search for homology throughout the genome is rate limiting [53,54], and argued that HR efficiency might be predicted *in silico* based on a locus' position. However, others have shown that subtelomeric DSBs in yeast are preferentially repaired by BIR, rather than gene conversion, despite the presence of homology on both sides of the break [55]. Increasing the clustering of

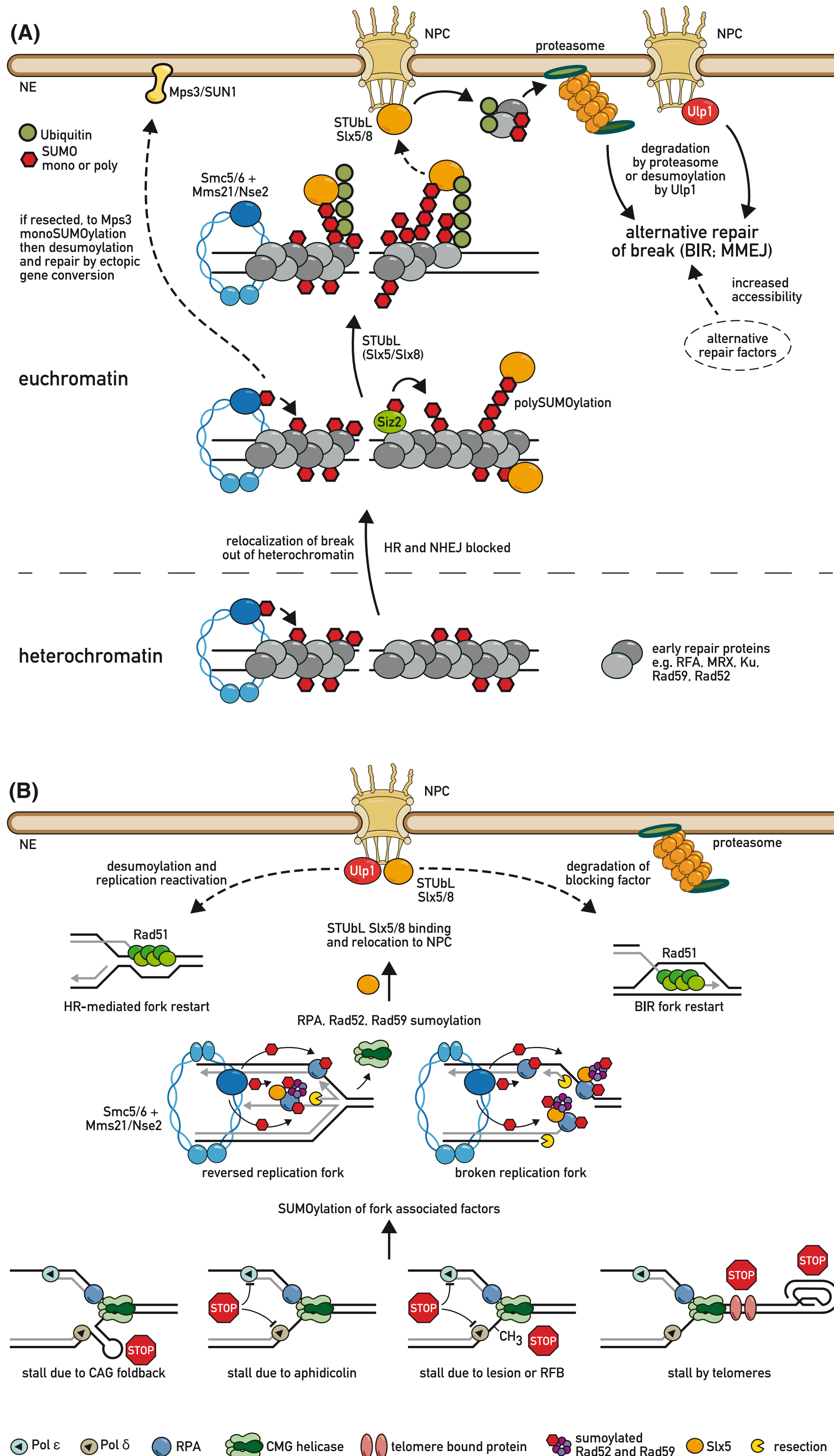

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Fig. 1. Pathways of repair and their relationship to Small Ubiquitin-related MOdifier (SUMO)-induced relocation to NPC and other nuclear envelope complexes. (A) Double-strand breaks (DSBs) can occur in heterochromatin or euchromatin, and the chromatin context of the damage influences the preferred pathway of repair. Smc5/Smc6 and mono-sumoylation by MMS21/Nse2 (dPIAS in flies) shift DSBs out of heterochromatin to enable repair by recombination-mediated mechanisms, particularly in S/G2 phases of the cell cycle. Recruitment of additional SUMO E3 ligases to the DSBs promotes poly-SUMO ligands that facilitate SUMO-targeted E3 ubiquitin ligases (STUbL) (Slx5/Slx8) binding and STUbL-dependent relocalization of the DSB to the nuclear pore complex (NPC). At the NPC, proteins are ubiquitylated and degraded by the proteasome to allow alternative repair factors to bind the break. Alternatively, damage-associated factors may be desumoylated and reactivated, as in the case of fork restart (see [36] and panel B). In budding and fission yeast, mono-sumoylated factors found at DSBs can also lead to damage relocation to SUN-domain proteins like Mps3, an inner nuclear envelope SUN-domain factor (Sad1 in *S. pombe*). This latter event is independent of STUbL (Slx5/Slx8) interactions, but the shift of damage to Mps3 still depends on Smc5/Smc6-Mms21 mono-sumoylation. Persistent DSBs or blocked ends that have no template for repair in budding yeast undergo a similar pathway, as described in the text. (B) In the case of stalled or collapsed replication forks, a somewhat similar scenario plays out, although recent work has clearly shown that in some cases the proximity to the NPC-associated Ulp1 triggers desumoylation of factors required for recovery or reactivation of replication-dependent pathways of repair [37,38], alongside the potential clearance of factors that might be blocking the strand invasion process [36]. Among the likely targets for desumoylation during fork restart are the mini-chromosome maintenance (MCM) complex and DNA Polε, while Rad52 and Rad51 are implicated in strand invasion events. See text for details and references. In the case of telomeric damage, the outcome also depends on strand invasion through break-induced replication (BIR), which generates Type II survivors through telomere end duplication. In the case of replication fork collapse or arrest, recombination can trigger a full fork restart in a Rad51-dependent manner. Rad51, unlike Rad52, may be activated by desumoylation, based on studies in *S. pombe* [38]. This figure is based on a figure in the excellent review by Whalen and Freudenreich [36] and summarizes work described in [37] and [38].

telomeres does increase HR-dependent gene conversion rates, but studies of chromatin context argued that the increase depended more on the chromatin context of the break than on the spatial proximity [55]. Consistently, the enhanced mobility of a DSB, which increases the likelihood of encountering an ectopic template, was found to be less important than the template accessibility for the efficiency of strand invasion [22], again suggesting that chromatin context and template accessibility are rate-limiting. Template accessibility is governed by nucleosome density [22], which in turn is controlled following checkpoint activation by damage-recruited Ub ligases and proteasome-mediated histone degradation [56]. In conclusion, while damage-clustering at pores may help fine-tune the homology search, repair factor turnover and pathway choice are more likely influenced by the fact that NPCs bring together STUbLs, the SUMO protease Ulp1, and proteasomes, to unblock ends and generate accessible templates [57]. Below we review data on the role of the SUMO machinery in positioning DNA DSBs, collapsed replication forks, and DNA-protein crosslinks (DPCs) at the NPC for appropriate repair.

The role of SUMO and NPCs in DSB repair

As stated above, the relocation of an induced DSB at the euchromatic *MAT* locus in yeast occurs in a SUMO-dependent manner [19,42] and both the Slx5/Slx8 STUbL and its interaction partner at the NPC, Nup84, contribute to both localization and repair [19]. Budding yeast *slx5*- and/or *slx8*-deficient mutants accumulated DNA recombination foci and gross chromosomal rearrangements [19]. Not surprisingly, abruptly shortened yeast telomeres, which resemble DSBs, also shift to the NPC [58,59]. Prior to STUbL binding, proteins at the DSB are modified by both SUMO E3 ligases Mms21 and Siz2 [44], with Mms21 monosumoylating and Siz2 generating poly-SUMO chains. This modification recruits Slx5/Slx8 and stimulates its ubiquitination activity [60]. While a key Slx5/Slx8 target is the HR repair protein Rad52, other damage-binding factors, including Rad59, Sae2, Ku, cohesion, and the SMC-like Mre11/Rad50/Xrn2 (MRX) complex, may be targeted for degradation as well (discussed in [36,61]).

A consensus model based on these diverse observations in yeast and higher eukaryotes would be that sequential mono- and poly-sumoylation generates binding sites for STUbLs, which together with either a conformational change or additional signal, favors break relocation to the NPC (Fig. 1A). The Slx5/Slx8 STUbL complex clearly helps relocation to nuclear pores, as Horigome *et al*. showed that in G1-phase cells the binding of a lexA-Slx5 fusion protein alone to a nondamaged site was sufficient to shift the site to the NPC [44]. Sumoylation initially seems to inhibit HR, but it allows ubiquitination by Slx5/Slx8, which in turn leads to the degradation of certain modified proteins, to enable either ectopic recombination, BIR, or HR to occur (Fig. 1A). In some cases, poly-sumoylation and Slx5/Slx8 may not be needed; notably in S/G2 phase resected DSBs are localized to the inner nuclear membrane SUN domain proteins, Mps3 or Sad1, in a Mms21-mediated mono-sumoylation-

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dependent manner, as discussed above (Fig. 1A). Other potential cooperative signals for translocation to the nuclear envelope may be phosphorylation events catalyzed by the ATR homolog, Mec1, or the deposition of the histone variant H2A.Z by the SWR remodeler complex [45]. The presence of proteasomes [57] and of the SUMO protease Ulp1 at or near pores [62,63] allows modified substrates to be either degraded or desumoylated, enabling more than one repair outcome (Fig. 1).

Special roles for SUMO in rDNA stability

As described above, breaks in the rDNA resemble DSBs in heterochromatic repeats in many ways, yet much of the rDNA is not heterochromatic. As mentioned above, the Lisby laboratory initially observed that a DSB in the rDNA relocates out of the nucleolus prior to processing for repair by HR [31], in a manner dependent on Rad52 sumoylation and the activity of the Smc5/Smc6-associated SUMO ligase, Mms21. Mutations that suppress these activities led to a loss of Rad52 focus relocation, and provoked hyper-recombination and the excision of extrachromosomal rDNA circles [31]. It was unclear whether the sumoylation itself promoted DSB relocation, or simply inhibited HR transiently, to allow damage to shift position through another mechanism. In a more recent study, Horigome et al. showed that replication-dependent damage in the rDNA associates with the NPC in a Tel1 checkpoint kinase-dependent manner, requiring only Mms21, and not Siz2 (i.e., mono- and not poly-sumoylation) [64]. The authors suggested that this relocation reflects the conversion of the replication fork block to a break. As observed earlier, interference with damage relocation induced repeat instability in the rDNA [64], thus both replication-associated damage and discrete DSB events in the rDNA require SUMO-dependent relocation.

SUMO in the repair of replication fork collapse

The replication fork contains a number of key enzymes that become sumoylated in response to replication fork damage [15,18] (Fig. 1B). The Zhao laboratory specifically examined the impact of such sumoylation events and found that sumoylation of the hexameric minichromosome maintenance (MCM) replicative helicase blocks the phosphorylation events that are necessary to initiate DNA replication [65]. Since MCM activity is necessary for fork restart, desumoylation of MCMs may regulate fork restart at collapsed or stalled forks. Similarly, the catalytic subunit of the leading strand DNA polymerase ε is specifically sumoylated in response to replication fork blockage [66].

Sumoylation has also been implicated in recovery from damage during DNA replication, when fork progression is stalled by triplet CAG repeats. The ensuing damage, if not appropriately repaired, drives repeat number variation and a number of human genetic diseases. The Freudenreich laboratory has shown that forks blocked at CAG repeat shift to the NPC in a Slx5/Slx8-dependent manner, much like persistent DSBs [67], reviewed in [36]. Interestingly, forks lacking repeats that are stalled by HU did not associate with the NPC [19], suggesting that paused forks are distinct from collapsed or folded-back structures. In the context of CAG-induced fork arrest, Rad52 is sumoylated, ubiquitinated and, after relocation to the pore, degraded by the proteasome system. Its degradation was followed by fork restart and a decrease in aberrant recombination [67]. A more recent paper showed as well that relocalization of CAG-induced fork collapse to the NPC required Mms21 SUMO ligase activity, as shown earlier for persistent DSBs [37]. In the context of replication fork damage, it is possible that juxtaposition to the NPC may allow Ulp1 to desumoylate MCMs, for example, enabling fork restart [36] (Fig. 1B).

Indeed, it is likely that the trigger for relocation to the NPC is not one specific sumoylation event, but rather an accumulation of multiple sumoylation targets at collapsed forks or processed DSBs. Replication protein A, Rad52, and Rad59 are all targets of sumoylation induced by ssDNA and checkpoint activation, and each may contribute to damage relocation, but then be subsequently degraded (reviewed in [36]). Rad51, on the other hand, is needed for both fork restart and strand invasion during recombination-mediated repair pathways, thus Rad51 would need to be stabilized rather than degraded. In support of such a model, a recent study in *S. pombe* by Kramarz *et al*. [38] used a natural replication fork barrier (RFB) and found that forks arrested at the RFB shifted to nuclear pores after Rad51 loading in a manner dependent on poly-sumoylation by the fission yeast E3 SUMO ligase Pli1 [38]. These authors present evidence that recruitment to the NPC allowed desumoylation of multiple targets by the SUMO protease Ulp1, triggering Rad51-dependent DNA synthesis and fork restart [38] (reviewed in [36] and Fig. 1B). In conclusion, the processing of collapsed forks may require a cycle of sumoylation and desumoylation facilitated by spatial relocation, although in other contexts (or species) pore association appears to trigger the degradation of factors that impede end processing. What triggers desumoylation as opposed to ubiquitination by a STUbL and degradation, is still unclear.

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The common take-home from these diverse systems is that poly-sumoylation and its recognition by Slx5/Slx8 promote damage relocalization to the NPC, while mono-sumoylation can delay inappropriate repair or processing of single-stranded templates, possibly by association with the Mps3/Sad1 complex. NPC association brings the lesion and its associated factors to the pore-bound SUMO protease [62,63], as well as pore-proximal proteasomes [57]. Whereas the ubiquitination by STUbLs may trigger the degradation of cohesin and end-blocking factors like MRX, other factors and enzymes that are needed for recovery, such as MCMs and DNA pol ε, or Rad51 in *S. pombe*, may be desumoylated and reactivated [37,38]. The common event in the regulation of these diverse repair events appears to be sumoylation-mediated relocation, even though it can lead to different outcomes for the sumoylated factors [58,68,69].

The role of SUMO in DNA-protein crosslink repair

Interesting functional links have also been established between sumoylation and DPC repair, which is a major challenge to genome integrity. DNA-protein crosslinks reflect a covalent bond between a protein and a DNA strand, that interferes severely with essential cellular processes such as DNA replication or transcription. Lack of DPC removal can lead eventually to cell death [70–73]. Because endogenous DPCs form under normal physiological conditions, carefully controlled DPC repair mechanisms have evolved.

Two pathways lead to DPCs: they can arise either from unspecific chemical crosslinking (non-enzymatic DPCs) or from the trapping of abortive enzymatic reactions (enzymatic DPCs), the most common of which involve involve DNA topoisomerases. The most frequent non-enzymatic sources of DPCs result from exposure to ionizing radiation, UV light, or substances such as reactive aldehydes [70], while Type I topoisomerases (Top1 in yeast) form covalent complexes between one strand of the DNA and a tyrosine residue in the enzyme's active site as they alleviate torsional stress during transcription or replication [74–76]. This reaction intermediate, known as Top1 covalent complex (Top1cc), allows DNA rotation, which releases tortional stress. The last step of the reaction is that of DNA religation, failure of which irreversibly traps the enzyme in a Top1cc intermediate. Such covalent complexes occur *in vivo* as part of the enzymatic cycle, but also accumulate upon exposure to chemotherapeutic agents such as camptothecin (CPT) or its derivatives, as camptothecin sits in the Top1 active site preventing the final religation step of repair and enzyme release [77].

Because CPT is used in chemotherapy, repair mechanisms dedicated to Top1cc clearance have been extensively studied. The repair involves the tyrosyl-DNA-phosphodiesterase Tdp1 that hydrolyzes the bond between the Top1 enzyme and DNA [78,79]. Degradation of the protein attached to DNA requires the metalloprotease Wss1 (Weak suppressor of *smt3*) in yeast (SPRTN in mammals) [80–84]. Yeast cells lacking both Wss1 and Tdp1 are defective in Top1cc repair, are very sensitive to CPT-induced damage, and show slow growth even in the absence of CPT. Wss1 acts not only on Top1cc structures, but on a variety of protein-DNA covalent links. Nonetheless, Wss1 is a DPC-dedicated protease that recognizes sumoylated targets surrounding the DPC through its SIM motifs. Finally, Wss1 functions in association with the AAA ATPase/segregase Cdc48 (p97 in mammals), which is targeted to DPCs through adaptors such as Doa1 or Ubx5, which bind Ub- or SUMO-modified proteins. The AAA ATPase/segregase Cdc48 contributes to the processing of the DNA adducts and is able to unfold ubiquitinated proteins for removal or degradation [39,83,85,86].

While these initial steps of Top1cc repair are fairly well understood [76,87,88], it remained unclear to which extent other repair components deal with DPCs. To identify additional pathways in yeast, a SATAY transposon screen [89] was performed in the slow-growing *tdp1Δ wss1Δ* mutant strain deficient in Top1cc processing. Among the hits that were functionally more important in *tdp1Δ wss1Δ* as opposed to wild-type cells was *DDI1* (DNA damage inducible-1). Ddi1 is a yeast aspartic protease implicated in DPC proteolysis, as well as the removal of tightly bound proteins from chromatin [90]. Ddi1 has proteolytic activity *in vitro* on substrates with very long Ub chains, but unlike Wss1, does not have a SIM [91]. The role of Ddi1 in DPC metabolism was further characterized by using the inducible Flp-nick system. The Flp-nick system is based on a recombinase point mutant, Flp-H305L, which binds an integrated flippase recognition target (*FRT*) locus and generates a single-strand nick with covalent recombinase crosslink, mimicking a Top1cc [92]. In the absence of Tdp1 and Wss1, Ddi1 was efficiently recruited to the *FRT* site containing the Flp-cc and promoted DPC processing. Genetic analysis then showed that Wss1, Ddi1, and the 26S proteasome act in parallel to provide resistance to a range of DPC-inducing agents [90].

Interestingly, the *tdp1Δ wss1Δ* SATAY transposon screen also identified a number of suppressors of this mutant's slow-growth phenotype. Besides loss of *TOP1*, the loss of *UBX5* turned out to be a very strong suppressor in the *tdp1Δ wss1Δ* background.

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Indeed, a recent study showed that in the absence of Wss1, the Cdc48-Ubx5 complex gets trapped on the Flp-cc *FRT* locus and prevents access to alternate repair pathways, such as one involving Ddi1 [86]. Other strong suppressors of *tdp1Δ wss1Δ* were linked to sumoylation, including the Siz2 SUMO ligase and the STUbL subunits Slx5 and Slx8, which, as described above, mediate damage relocation to NPCs, conferring SUMO-dependent ubiquitination. Several NPC components, such as Nup60 and Mlp1, which are responsible for Ulp1 anchoring at the pore, were also recovered as suppressors [93]. These hits implicate SUMO metabolism mediated by Ulp1, Siz2, Slx5, and Slx8 in the control of the repair of Top1cc or analogous DPC lesions.

Genetic analysis further revealed that although SUMO promotes Top1cc processing in the absence of Tdp1, it has an inhibitory role if cells additionally lack Wss1. In the *tdp1Δ wss1Δ* double mutant, the E3 SUMO ligase Siz2 sumoylates targets in the vicinity of the DPC, inhibiting alternative DPC repair mechanisms, such as that mediated by Ddi1. Thus, sumoylation and its turnover coordinates the available repair pathways to facilitate DPC repair. Intriguingly, the genetically dominant *ΔN-ulp1* SUMO protease mutant, which lacks the N-terminal pore-interacting domain but remains catalytically active, significantly rescued the *tdp1Δ wss1Δ* strain slow growth. This may reflect the fact that an unanchored Ulp1 strongly decreases Siz2 protein levels, and thereby reduces sumoylation [93]. As one might predict, the growth of the *tdp1Δ wss1Δ siz2Δ* strain was strongly dependent on Ddi1. In other words, sumoylation by Siz2 and ubiquitination by the STUbL Slx5/Slx8, which both accumulate at DPCs, become toxic in the context of *tdp1Δ wss1Δ* because they interfere with the alternative Ddi1 repair pathway (Fig. 2).

These genetic and molecular observations implicate SUMO and Ub post-translational modifications in orchestrating DPC repair pathway choice. Given the important role of the NPC in regulating SUMO modifications and in the repair of various types of DNA damage, as well as the impact of pore protein

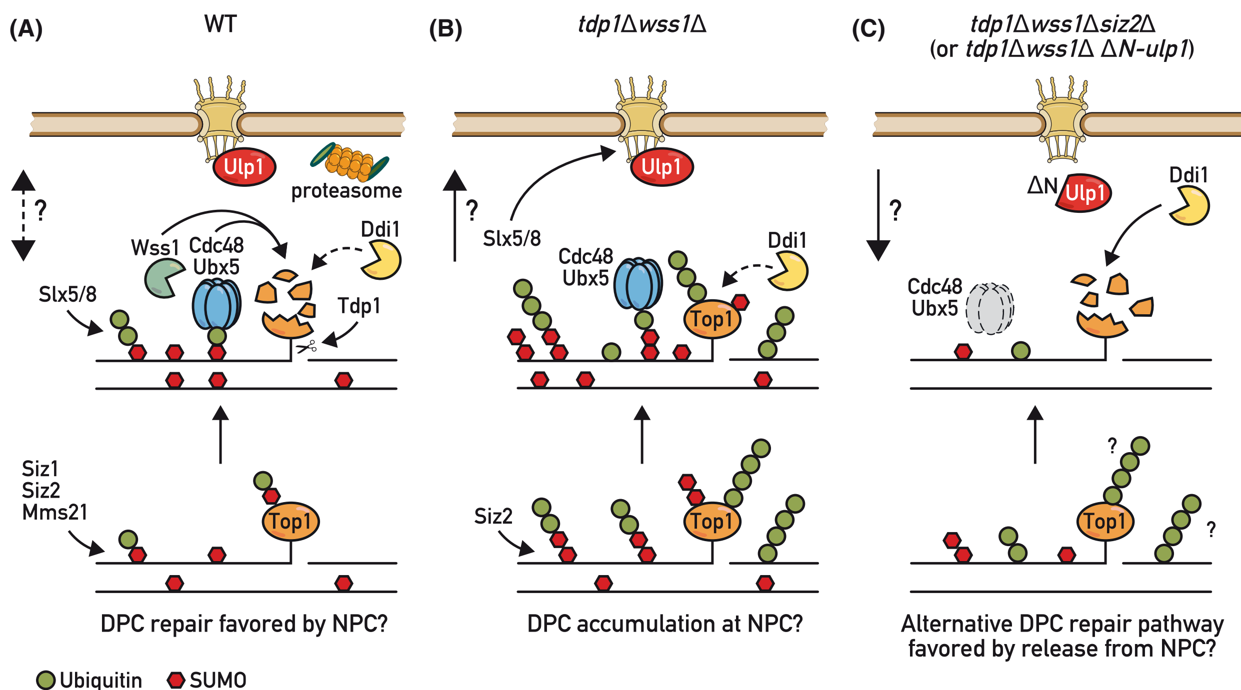

Fig. 2. Hypothetical link between Small Ubiquitin-related MOdifier (SUMO), Double-strand breaks (DPC) repair, and the nuclear pore complex (NPC). (A) In WT cells, both sumoylation and ubiquitination near a DPC contribute to the recruitment of the Cdc48-Ubx5 ATPase and the Wss1 metalloprotease promoting digestion of the protein adduct. The figure shows the modifications in the vicinity of a Top1cc, which will promote proteolysis followed by hydrolysis of the protein-DNA bond by Tdp1. An open question is whether these reactions may be regulated or stimulated by the NPC environment. (B) In *tdp1Δwss1Δ* strains, Cdc48-Ubx5 accumulates at the DPC site [86], where proteins become heavily sumoylated by Siz2 and ubiquitinated by Slx5/Slx8 [93]. These modifications may promote DPC trapping at the NPC and prevent access to alternate repair pathways such as Ddi1. (C) In *tdp1Δwss1Δsiz2Δ* or *tdp1Δwss1Δ ΔN-ulp1*, both sumoylation and ubiquitination are reduced, potentially releasing the DPC from the pore and allowing access to alternate repair pathways such as Ddi1, which recognizes substrates with very long ubiquitin (Ub) chains deposited by a still undefined Ub ligase [91].

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mutations on DPC repair, it would be interesting to know how DPC metabolism is influenced by the lesion's subnuclear localization. So far, there is only one example that reports a physical link between DPC repair and the nuclear periphery in mammalian cells [94]. This study shows that a cofactor to the p97/Cdc48 AAA ATPase/segregase called TEX264 is enriched at the nuclear periphery and associates with replication forks. TEX264 recognizes both non-modified and SUMO-modified TOP1 and promotes TOP1cc repair by recruiting p97/Cdc48 and SPRTN to the DPC [94]. TEX264 is a cofactor specific for TOP1cc repair, and it remains unknown if the repair of other types of DPCs also depends on cofactors found at the nuclear periphery.

It would be interesting to check whether the increased sumoylation and ubiquitination observed at the *FRT* site upon Flp-cc induction in *tdp1Δ wss1Δ* promotes DPC relocalization to the NPC in yeast, thereby preventing the access of other repair pathways. Loss of Siz2 or delocalization of Ulp1 from the NPC likely reduces DPC site sumoylation, as well as Slx5/Slx8-dependent ubiquitination, thereby preventing DPC accumulation at the NPC and favoring its resolution by Ddi1 (Fig. 2).

The role of SUMO in transcriptional regulation

The nuclear periphery has long been considered to be a zone that antagonizes transcription, given that silent chromatin concentrates at the nuclear envelope in yeast [50], as in nearly every differentiated cell-type found in multicellular organisms [95]. In contrast, NPCs preferentially associate with highly induced genes, not only in yeast, but in flies and worms [96–100]. A number of promoter-associated factors have been implicated in the relocalization of activated genes to the NPC, including the coactivator SAGA and components of the Mediator complex, but also factors recruited during transcription and mRNA biogenesis, such as the THO/TREX complex or the export receptor Mex67 [101].

Excellent genetic studies suggested that the NPC contributes to optimal gene activation by controlling the localization of Ulp1 in yeast [11,102] (Fig. 3). It was shown that loss of nucleoporins such as Mlp1/Mlp2 or Nup60, which are all involved in Ulp1 anchoring [62,63], resulted in faster kinetics of *GAL1* gene activation when cells were shifted from glucose to galactose. Deletion of the Ulp1 N-terminal domain (*ΔN-ulp1*), which mediates Ulp1-NPC anchoring, resulted in a similar phenotype, while artificial anchoring of Ulp1 to the NPC suppressed the rapid gene induction provoked by *mlp1* and *mlp2* deletion. This

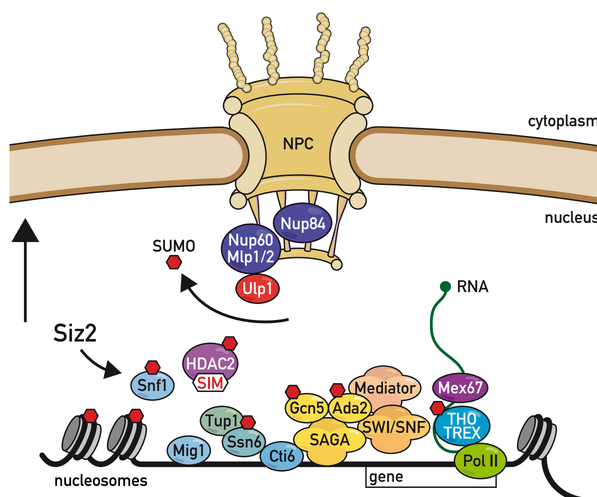

Fig. 3. Link between Small Ubiquitin-related MOdifier (SUMO), nuclear pore complex (NPC), and transcription regulation. In budding yeast, *Drosophila*, and a few mammalian systems, it has been shown that active genes are anchored at NPCs through key transcriptional complexes such as SAGA, TREX, and—in yeast—the transcription repressors Tup1 and Ssn6 (reviewed in [50,95–100]; see text for primary references). Cycles of sumoylation/desumoylation, respectively, by Siz2 and NPC-associated Ulp1 of a number of gene-associated factors (repressors and transcription regulators) participate in NPC gene anchoring and optimal gene regulation [11,102,103].

argued that the major role of the pore basket in gene induction is its ability to bind and sequester the SUMO protease Ulp1 [102]. The faster gene activation likely stems from the fact that a number of gene-associated transcriptional repressors, notably Tup1 and Ssn6, require sumoylation for their repressive function, and therefore require desumoylation for their inactivation. Delocalization of Ulp1 would reduce sumoylation globally and in turn upregulate transcription. Consistently, this phenocopies specific Ssn6 KR mutations that prevent sumoylation [102].

It is noteworthy that the *GAL1* gene does not relocate to the NPC upon activation when Ulp1 is not present at the NPC (either in *mlp1Δ mlp2Δ* or *ΔN-ulp1*). This argues that Ulp1 protein may play a direct role in anchoring the activated *GAL1* gene with the NPC [102]. Spatial proximity to the pore has also been proposed to enhance efficient mRNA export, given that heat shock loci, which are rapidly induced and highly transcribed, are found associated with the NPC both before and after activation, in both flies and *C. elegans* [104,105]. For heat shock loci, which depend on SAGA for induction, there is an associated DUB (deubiquitinating complex) that is important for pore basket association [105]. Finally, GCN5, a core component of SAGA, is also sumoylated, although the relevance in this context is unclear [106].

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The importance of the NPC as the platform for optimizing inducible gene expression was convincingly demonstrated for the inducible gene *HXK1* already in 2006 [98]. A more recent study focuses on the inducible *INO1* gene, and has further dissected the link between gene expression and recruitment to the NPC. Again, sumoylation by Siz2 of proteins bound to the promoter and gene body was shown to be required for *INO1* gene recruitment to the NPC where the transcription initiation complex interacted with Ulp1. The presence of catalytically active Ulp1 at the pore further stabilized *INO1* anchoring and efficient mRNA production [103]. Together, these observations support the view that optimal regulation and expression of inducible genes such as *INO1*, *HXK1*, or *GAL1* depends on cycles of sumoylation and NPC-linked desumoylation, which affect a range of transcription factors (Fig. 3). We conclude that the NPC primarily impacts inducible gene expression, at least in yeast, by serving as a platform for SUMO binding and proteolysis.

The role of SUMO at telomeres

The tethering of full-length natural telomeres at the nuclear periphery is also SUMO-dependent in both yeast and *C. elegans*, even without DNA damage checkpoint activation. Telomere anchoring to the nuclear periphery in budding yeast is mediated by at least two pathways, one which depends on subtelomeric silent chromatin and one that does not [107–109] (Fig. 4). Budding yeast silent chromatin depends on the binding of a repressive complex of Sir2, Sir3, and Sir4 (the SIR complex) to nucleosomes, notably Sir2-Sir4 to the deacetylated tail of histone H4, and Sir3 to the interface between two adjacent nucleosomes (reviewed in [117]). The spreading of silent chromatin is triggered by the deacetylation of histones by Sir2-Sir4 and by Sir4's ability to recruit Sir3, which then binds nucleosomes [117]. At telomeres, the nucleation of silencing initiates from the cluster of binding sites for Rap1 found in the terminal $(TG_{1\text{-}3})n$ repeat, as Rap1 binds both Sir3 and Sir4 (Fig. 4). The perinuclear anchoring of silent chromatin is mediated through the affinity of Sir4 for Esc1, a prenylated protein associated with the inner nuclear membrane [107,115]. Sir4 also binds the Yku70/Yku80 dimer (hereafter yKu), which binds the inner nuclear membrane SUN domain factor, Mps3 (Fig. 4) [110,111]. Given that the natural chromosome end also binds yKu, which helps recruit telomerase, both Sir4 and yKu play a role in proper telomere length maintenance by telomerase. As indicated in Fig. 4, the nuclear envelope tethering of the telomere through yKu-

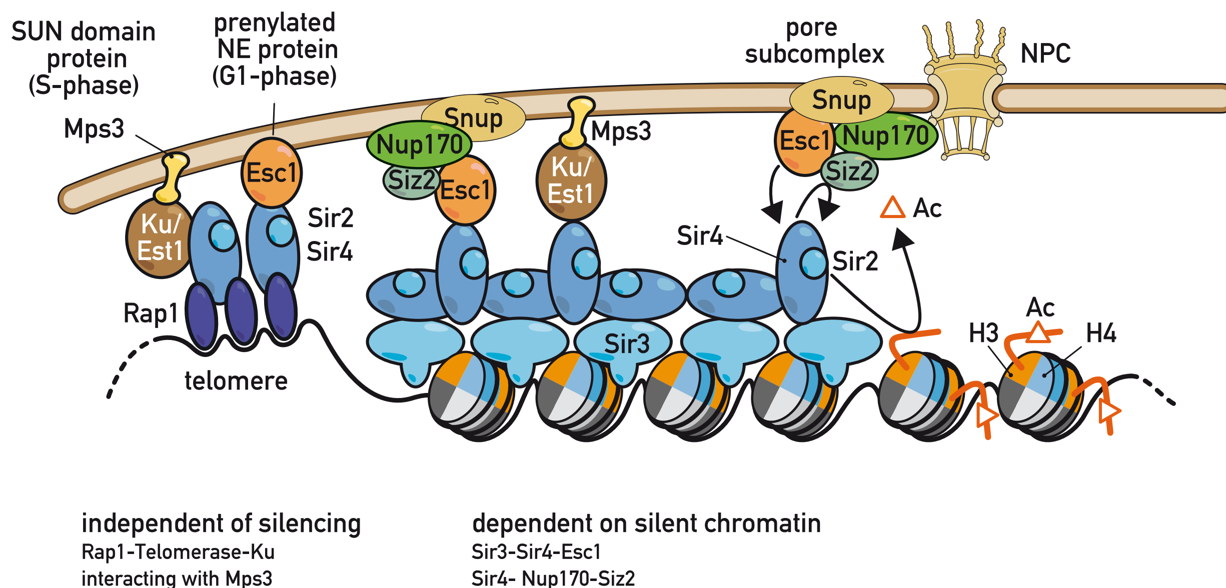

Fig. 4. Two pathways tether silent chromatin in yeast. Telomeres are tethered to the yeast's inner nuclear envelope by two pathways that reflect interactions either with subtelomeric SIR-bound silent chromatin, or the end-binding complex yKu. The latter is mediated by yKu interaction with the SUN domain inner nuclear envelope factor Mps3 (SUN1 homolog). The Rap1-Sir4 binding at telomeric repeats also interacts with yKu, forming a second bridge to telomerase (see Fig. 5). The latter link to the nuclear envelope is independent of SIR-mediated silencing, while the association of silent chromatin depends on the interaction of Sir4 with Esc1, a prenylated component of the inner nuclear envelope. Esc1 and Sir4 are also associated with a subcomplex of the nuclear pore (the SNUP complex) which contains Nup170, Nup192, Nup157, Nup188, Nup84, Nup133, and Nup145C, but which lacks a number of other core NPC components [114]. Loss of Nup170-Sir4 interaction compromises telomere association with the nuclear envelope, as does loss of Esc1 or Sir4. Loss of Nup170 also compromises telomeric silencing partially, as does loss of Esc1 [115,116].

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Mps3 is primarily active in S phase, while tethering through Sir4-Esc1 is dominant in G1.

Recently, the Wozniak laboratory identified a pore subcomplex containing NPC-component Nup170, along with Esc1, Sir4, and Siz2, that mediates Sir4 and silent chromatin interaction with the nuclear envelope [114]. The complex, called SNUP, harbors Nup170 as the key Sir4-binding factor, along with Nup192, Nup157, Nup188, Nup84, Nup133, and Nup145C, although it lacks many other core NPC components [114]. Consistent with earlier findings of the Gasser laboratory on Sir4-Esc1 anchoring of telomeres, the Nup170-Sir4-Esc1 axis for telomere anchoring is especially important in G1-phase cells [116], and the disruption of *nup170* shows only partial derepression of subtelomeric reporter genes, much like *esc1* disruption [107,115,116]. Whereas the deletion of *sir4* completely ablates subtelomeric silencing, one must disrupt both the genes encoding Sir4 and yKu to release telomeres from their association with the nuclear envelope [108,118–120]. This is due to the existence of a parallel, silencing-independent anchoring pathway, which depends on other factors recognizing the chromosome end.

This silencing-independent tethering pathway depends on the sumoylation-dependent interaction of the yeast telomerase subunits Est1, Est2 and the RNA subunit of telomerase, Tlc1, with the SUN-domain protein, Mps3 [110,111]. Est1 itself binds the yKu DNA end-binding complex through the Yku80 subunit [111], and yKu80 binds Sir4 in a sumoylation-enhanced manner. The SUMO E3 ligase Siz2 modifies Yku70, Yku80, and Sir4 *in vivo* [110,111]. Thus, the loss of the SUMO ligase, Siz2, significantly compromises telomere anchoring through two pathways: it not only compromises the SIM-SUMO ligand interactions on the silencing-independent anchoring pathway, but as an integral component of the SNUP complex, Siz2 directly links Esc1, Sir4, and Nup170 to a nuclear pore subcomplex in G1 [114] (Fig. 5).

Sumoylation was not only shown to be necessary for telomere anchoring to the nuclear envelope in yeast, but also in *C. elegans* [110,121]. Using genetic means, it was shown in early *C. elegans* embryos that telomere clustering at the nuclear periphery requires the nuclear envelope protein SUN-1, the telomere-specific single-strand binding protein POT-1, and the SUMO ligase GEI-17, which is equivalent to Siz2 in yeast [121]. This mode of end tethering varies through worm development, suggesting that even though conserved across species, the role of SUMO in the sub-nuclear localization may be differentiation-stage specific.

Sumoylation has also been invoked as a player in the repression and localization of heterochromatin in mammals, albeit through a very different pathway.

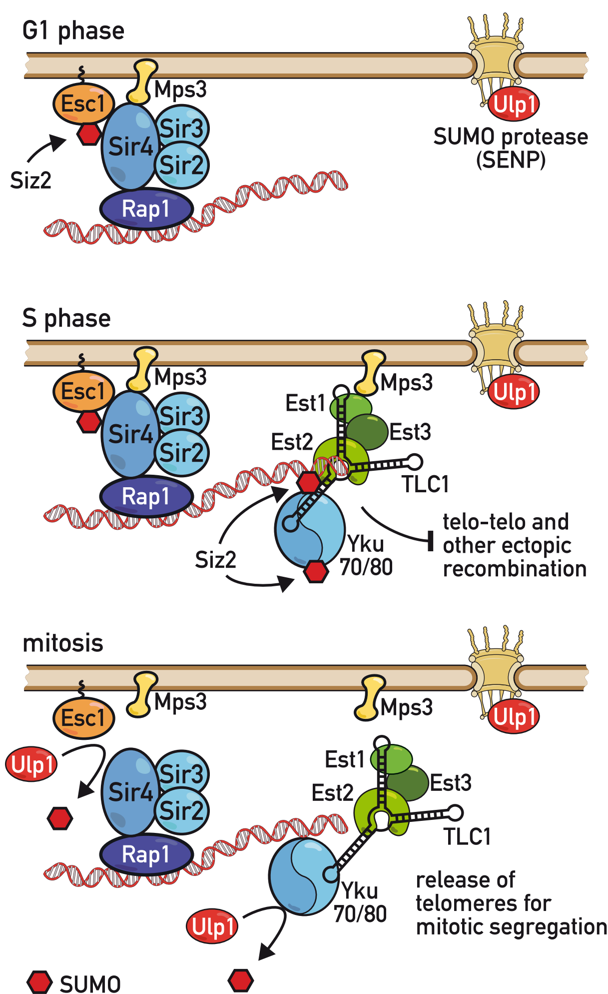

Fig. 5. SUMO contributes to regulated yeast telomere anchoring: Siz2 sumoylation and Ulp1 desumoylation alter telomere positioning through the cell cycle. The three panels illustrate the roles played by SUMO and/or Siz2 in the telomere anchoring pathways other than those dependent on silent information regulator (SIR)-mediated silencing (Fig. 4) at different stages of the cell cycle in the budding yeast, *S. cerevisiae*. In G1 phase, the Sir4-Esc1 interaction is key, which can also include the SNUP complex of Nup170 and the subset of NPC components described in Fig. 4. Sir4 itself is a target of sumoylation by Siz2 [110,111]. In S phase, the SUN1 homolog Mps3 also helps tether telomeres, independent of silent chromatin, through the yeast telomerase subunits Est1, Est2, and Tlc1 (see text). Yku70 and Yku80 are multiply sumoylated. The presence of this complex reduces ectopic recombination events at the telomere as it becomes uncapped for replication. In mitosis, as proposed by Ferreira et al. [110,111], Ptak et al. [112], and Saik et al. [113], desumoylation mediated by Ulp1 at the NPC may release telomeres to facilitate end replication and segregation of telomeres. Mitotic phosphorylation is then thought to precede re-sumoylation and telomere reattachment for the next cell cycle [112].

Heterochromatin protein 1, the major ligand of the repressive histone H3K9me2/3 mark is sumoylated and its loading appears to be facilitated by the propensity

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of the H3K9 histone methyltransferase SUV39H1 to load this modified form of HP1 [122]. The SUMO protease, SENP7, then plays a key role in stabilizing HP1 on chromatin presumably by binding the desumoylated form of the repressor HP1 once it is loaded on chromatin [122–124]. Nonetheless, the tethering of the 1–167 domain of SUV39H1 to pericentric heterochromatin accelerates the *de novo* targeting of HP1α to these domains, but only if SUV39H1 retains its ability to bind the UBC9 SUMO ligase. It remains unclear whether sumoylated HP1 or its ligands help target heterochromatin to lamin A, which contains a SIM domain [125]. It is thus possible, but remains unproven, that mammalian heterochromatin anchoring to the lamina involves sumoylation.

The function of telomere tethering and the unusual STUbL Uls1 in end maintenance

In budding yeast, the inactivation of telomerase causes telomere erosion and induces relocation of the shortened telomere to the NPC [58,68,69]. In a compelling study, Churikov *et al*. reported that Siz2-dependent sumoylation of RPA at eroded telomeres triggers relocation to the NPC through the Slx5/Slx8 STUbL. Similar to Whalen *et al*., the authors speculate that the targeting to the NPC allows the desumoylation of eroded telomeres giving the telomere another chance for repair [58], and releases it transiently from the nuclear envelope. Consistently, Aguilera *et al*. propose a key role for the NPC in suppressing sister-chromatid recombination (SCR) of stalled forks at telomeres after telomerase inactivation [126]. This same release of telomeres through desumoylation occurs in late S/G2 phase in yeast, coincident with telomerase elongation and facilitating mitotic segregation (Fig. 5). Subsequent mitotic phosphorylation then enables resumoylation, presumably in late telophase/G1, possibly restoring telomere anchoring upon entry into G1 [112].

Combining the intriguing effect of telomeric repeats and DSB induction on chromatin dynamics and subnuclear positioning (reviewed in [58,68,69]), the behavior of an internal DSB that is flanked on one side by telomeric-like TG repeats was also investigated in budding yeast [61]. In contrast to a canonical induced DSB whose relocation to the pore would normally be Slx5/Slx8-dependent, relocation of the TG-flanked DSB was dependent on another yeast STUbL, Uls1, which in addition to containing SIMs and Ub ligase activity, harbors an AAA/ATPase remodeler activity. The recognition of poly-sumoylated Rap1 by Uls1 [127] is thought to trigger the ubiquitination and degradation of Rap1 at telomeres. This probably also occurs at break-associated internal TG repeats, as well. In addition to Rap1, the largest component of the MRX complex, Mre11, is known to be sumoylated in response to DNA damage. Moreover, at an induced DSB, Mre11 and Rap1 are both depleted in a Uls1-dependent manner at TG-embedded breaks [61]. It was proposed that Uls1 ubiquitinates MRX and possibly Rap1, leading to their degradation and thereby enhancing break mobility [22]. In line with this hypothesis, it was shown that *uls1*-deficient cells have reduced DSB movement, fewer translocations, and more NHEJ, consistent with the accumulation of MRX at the TG-flanked DSB [22].

Uls1 is an unusual member of the STUbL family because in addition to harboring Ub ligase activity and multiple SIM motifs, it contains a SNF2-like helicase domain related to nucleosome remodelers. The likely homolog to Uls1 is the human E3 Ub ligase SHPRH, which was identified as a tumor suppressor that helps prevent genome instability [128,129]. It is thought that SHPRH increases DNA damage tolerance or post-replication repair, which depends on the ubiquitination of PCNA [130]. In budding yeast, Uls1 seems to regulate the accumulation of cohesin and MRX [131,132], as the loss of Uls1 correlated with enhanced levels of cohesin and MRX at a DSB, and reduced its dynamic movement [22]. It is possible, that Uls1 controls the levels of cohesin (Scc1) and MRX (Rad50) at all processed breaks through SUMO-dependent ubiquitination and subsequent degradation or eviction. It remains to be explored if the binding of Uls1 antagonizes or accelerates Slx5/Slx8 recruitment, or if it modulates relocation to the NPC.

Both cohesin subunits (SMCs and Mre11) were shown to be a major target of sumoylation following DNA damage [18]. In addition, MRX itself helps recruit SUMO ligases, and sumoylated Mre11 appears to recruit Uls1 [18,61,133]. Clearly, the impact of SUMO- and Ub-dependent removal of natural ring-forming complexes like cohesin, Smc5/Smc6, or MRX at sites of damage will need to be examined more in depth. Such degradation events could both improve access for the recombination machinery and release the damage from local constraints to accelerate the homology search. Given the abundance of cohesin found around the centromere, it will be interesting to see if centromere-proximal breaks require cohesin degradation for efficient repair, and whether this requires the activity of Uls1 or SHPRH in mammals.

Perspectives

The role of SUMO in the repair of collapsed replication forks or DSBs that cannot be repaired by recombination with the sister chromatid, are of course only a subset of

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the many types of repair in which sumoylation and ubiquitination play a role. We have not elaborated on the role of SUMO in transcription-coupled UV-induced damage repair [134] nor in other repair pathways (reviewed partially in [135]). It is important to note that many sumoylation events in repair may not involve the spatial relocation of the damage to the NPC, or else, in mammalian cells, may involve relocation to PML nuclear bodies. Nonetheless, we have summarized the extensive evidence that supports a role of SUMO in the regulation of difficult-to-repair DSBs, breaks in repetitive DNA, and at protein-DNA adducts. It will be important to examine the hot spots of STUbL binding under DNA-damaging conditions, to identify the factors that recruit STUbLs and find other signals that contribute to the spatial segregation of DNA damage [136]. Another aspect that is understudied at present is the role of the Cdc48/p97 ATPase, an enzyme that was repeatedly implicated in clearing ubiquitinated substrates to enable appropriate repair. It remains unclear how this complex selectively recognizes its targets and processes them. What other reactions take place near the NPC, and is the crosstalk of Cdc48/p97 with the proteasome spatially constrained within the nucleus? Finally, one should not forget that histones themselves are evicted and degraded in an Ub-dependent manner in response to damage [137,138], and that it remains unknown whether sumoylation, STUbLs, or subnuclear positioning is involved in histone mobilization and/or degradation. These questions address but a few of the outstanding unknowns that are highly relevant to grasping how SUMO and Ub contribute to genome stability.

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References

1 Ciehanover A, Hod Y and Hershko A (1978) A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. *Biochem Biophys Res Commun* **81**, 1100–1105.
2 Dikic I and Schulman BA (2023) An expanded lexicon for the ubiquitin code. *Nat Rev Mol Cell Biol* **24**, 273–287.
3 Melchior F, Schergaut M and Pichler A (2003) SUMO: ligases, isopeptidases and nuclear pores. *Trends Biochem Sci* **28**, 612–618.
4 Ptak C and Wozniak RW (2017) SUMO and nucleocytoplasmic transport. *Adv Exp Med Biol* **963**, 111–126.
5 Horigome C and Gasser SM (2016) SUMO wrestles breaks to the nuclear ring's edge. *Cell Cycle* **15**, 3011–3013.
6 Hay RT (2013) Decoding the SUMO signal. *Biochem Soc Trans* **41**, 463–473.
7 Tatham MH, Jaffray E, Vaughan OA, Desterro JM, Botting CH, Naismith JH and Hay RT (2001) Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. *J Biol Chem* **276**, 35368–35374.
8 Stewart MD, Ritterhoff T, Klevit RE and Brzovic PS (2016) E2 enzymes: more than just middle men. *Cell Res* **26**, 423–440.
9 Tasaki T, Mulder LC, Iwamatsu A, Lee MJ, Davydov IV, Varshavsky A, Muesing M and Kwon YT (2005) A family of mammalian E3 ubiquitin ligases that contain the UBR box motif and recognize N-degrons. *Mol Cell Biol* **25**, 7120–7136.
10 Alhazmi N, Pai CP, Albaqami A, Wang H, Zhao X, Chen M, Hu P, Guo S, Starost K, Hajihassani O *et al.* (2020) The promyelocytic leukemia protein isoform PML1 is an oncoprotein and a direct target of the antioxidant sulforaphane (SFN). *Biochim Biophys Acta Mol Cell Res* **1867**, 118707.
11 Texari L and Stutz F (2015) Sumoylation and transcription regulation at nuclear pores. *Chromosoma* **124**, 45–56.
12 Matic I, van Hagen M, Schimmel J, Macek B, Ogg SC, Tatham MH, Hay RT, Lamond AI, Mann M and Vertegaal ACO (2008) In vivo identification of human small ubiquitin-like modifier polymerization sites by high accuracy mass spectrometry and an *in vitro* to *in vivo* strategy. *Mol Cell Proteomics* **7**, 132–144.
13 Dhingra N and Zhao X (2019) Intricate SUMO-based control of the homologous recombination machinery. *Genes Dev* **33**, 1346–1354.
14 Jackson SP and Durocher D (2013) Regulation of DNA damage responses by ubiquitin and SUMO. *Mol Cell* **49**, 795–807.
15 Zhao X (2018) SUMO-mediated regulation of nuclear functions and signaling processes. *Mol Cell* **71**, 409–418.
16 Parker JL and Ulrich HD (2012) A SUMO-interacting motif activates budding yeast ubiquitin ligase Rad18 towards SUMO-modified PCNA. *Nucleic Acids Res* **40**, 11380–11388.
17 Parker JL and Ulrich HD (2014) SIM-dependent enhancement of substrate-specific SUMOylation by a ubiquitin ligase *in vitro*. *Biochem J* **457**, 435–440.
18 Cremona CA, Sarangi P, Yang Y, Hang LE, Rahman S and Zhao X (2012) Extensive DNA damage-induced sumoylation contributes to replication and repair and

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acts in addition to the mec1 checkpoint. *Mol Cell* **45**, 422–432.

19 Nagai S, Dubrana K, Tsai-Pflugfelder M, Davidson MB, Roberts TM, Brown GW, Varela E, Hediger F, Gasser SM and Krogan NJ (2008) Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* **322**, 597–602.

20 Xiong L, Chen XL, Silver HR, Ahmed NT and Johnson ES (2009) Deficient SUMO attachment to Flp recombinase leads to homologous recombination-dependent hyperamplification of the yeast 2 microm circle plasmid. *Mol Biol Cell* **20**, 1241–1251.

21 Zhang C, Roberts TM, Yang J, Desai R and Brown GW (2006) Suppression of genomic instability by SLX5 and SLX8 in Saccharomyces cerevisiae. *DNA Repair (Amst)* **5**, 336–346.

22 Cheblal A, Challa K, Seeber A, Shimada K, Yoshida H, Ferreira HC, Amitai A and Gasser SM (2020) DNA damage-induced nucleosome depletion enhances homology search independently of local break movement. *Mol Cell* **80**, 311.e4–326.e4.

23 Marnef A and Legube G (2017) Organizing DNA repair in the nucleus: DSBs hit the road. *Curr Opin Cell Biol* **46**, 1–8.

24 Marnef A, Finoux AL, Arnould C, Guillou E, Daburon V, Rocher V, Mangeat T, Mangeot PE, Ricci EP and Legube G (2019) A cohesin/HUSH- and LINC-dependent pathway controls ribosomal DNA double-strand break repair. *Genes Dev* **33**, 1175–1190.

25 Schep R, Brinkman EK, Leemans C, Vergara X, van der Weide RH, Morris B, van Schaik T, Manzo SG, Peric-Hupkes D, van den Berg J *et al*. (2021) Impact of chromatin context on Cas9-induced DNA double-strand break repair pathway balance. *Mol Cell* **81**, 2216.e10–2230.e10.

26 Smith KS, Liu LL, Ganesan S, Michor F and De S (2017) Nuclear topology modulates the mutational landscapes of cancer genomes. *Nat Struct Mol Biol* **24**, 1000–1006.

27 Janssen A, Breuer GA, Brinkman EK, van der Meulen AI, Borden SV, van Steensel B, Bindra RS, LaRocque JR and Karpen GH (2016) A single double-strand break system reveals repair dynamics and mechanisms in heterochromatin and euchromatin. *Genes Dev* **30**, 1645–1657.

28 Chiolo I, Minoda A, Colmenares SU, Polyzos A, Costes SV and Karpen GH (2011) Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell* **144**, 732–744.

29 Ryu T, Spatola B, Delabaere L, Bowlin K, Hopp H, Kunitake R, Karpen GH and Chiolo I (2015) Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. *Nat Cell Biol* **17**, 1401–1411.

30 Tsouroula K, Furst A, Rogier M, Heyer V, Maglott-Roth A, Ferrand A, Reina-San-Martin B and Soutoglou E (2016) Temporal and spatial uncoupling of DNA double Strand break repair pathways within mammalian heterochromatin. *Mol Cell* **63**, 293–305.

31 Torres-Rosell J, Sunjevaric I, De Piccoli G, Sacher M, Eckert-Boulet N, Reid R, Jentsch S, Rothstein R, Aragon L and Lisby M (2007) The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. *Nat Cell Biol* **9**, 923–931.

32 Harding SM, Boiarsky JA and Greenberg RA (2015) ATM dependent silencing links nucleolar chromatin reorganization to DNA damage recognition. *Cell Rep* **13**, 251–259.

33 van Sluis M and McStay B (2015) A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage. *Genes Dev* **29**, 1151–1163.

34 Kruhlak MJ, Celeste A, Dellaire G, Fernandez-Capetillo O, Muller WG, McNally JG, Bazett-Jones DP and Nussenzweig A (2006) Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J Cell Biol* **172**, 823–834.

35 Lemaitre C, Grabarz A, Tsouroula K, Andronov L, Furst A, Pankotai T, Heyer V, Rogier M, Attwood KM, Kessler P *et al*. (2014) Nuclear position dictates DNA repair pathway choice. *Genes Dev* **28**, 2450–2463.

36 Whalen JM and Freudenreich CH (2020) Location, location, location: the role of nuclear positioning in the repair of collapsed forks and protection of genome stability. *Genes (Basel)* **11**, 635.

37 Whalen JM, Dhingra N, Wei L, Zhao X and Freudenreich CH (2020) Relocation of collapsed forks to the nuclear pore complex depends on Sumoylation of DNA repair proteins and permits Rad51 association. *Cell Rep* **31**, 107635.

38 Kramarz K, Schirmeisen K, Boucherit V, Ait Saada A, Lovo C, Palancade B, Freudenreich C and Lambert SAE (2020) The nuclear pore primes recombination-dependent DNA synthesis at arrested forks by promoting SUMO removal. *Nat Commun* **11**, 5643.

39 Twomey EC, Ji Z, Wales TE, Bodnar NO, Ficarro SB, Marto JA, Engen JR and Rapoport TA (2019) Substrate processing by the Cdc48 ATPase complex is initiated by ubiquitin unfolding. *Science* **365**, eaax1033.

40 Capella M, Mandemaker IK, Martin Caballero L, den Brave F, Pfander B, Ladurner AG, Jentsch S and Braun S (2021) Nucleolar release of rDNA repeats for repair involves SUMO-mediated untethering by the Cdc48/p97 segregase. *Nat Commun* **12**, 4918.

41 Chen B, Ge T, Jian M, Chen L, Fang Z, He Z, Huang C, An Y, Yin S, Xiong Y *et al*. (2023) Author correction: transmembrane nuclease NUMEN/

18733468, 2023, 22, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.14751 by Bcu Lausanne, Wiley Online Library on [09/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

ENDOD1 regulates DNA repair pathway choice at the nuclear periphery. *Nat Cell Biol* **25**, 1384.

42 Kalocsay M, Hiller NJ and Jentsch S (2009) Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Mol Cell* **33**, 335–343.

43 Oza P, Jaspersen SL, Miele A, Dekker J and Peterson CL (2009) Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. *Genes Dev* **23**, 912–927.

44 Horigome C, Bustard DE, Marcomini I, Delgoshaie N, Tsai-Pflugfelder M, Cobb JA and Gasser SM (2016) PolySUMOylation by Siz2 and Mms21 triggers relocation of DNA breaks to nuclear pores through the Slx5/Slx8 STUbL. *Genes Dev* **30**, 931–945.

45 Horigome C, Oma Y, Konishi T, Schmid R, Marcomini I, Hauer MH, Dion V, Harata M and Gasser SM (2014) SWR1 and INO80 chromatin remodelers contribute to DNA double-strand break perinuclear anchorage site choice. *Mol Cell* **55**, 626–639.

46 Swartz RK, Rodriguez EC and King MC (2014) A role for nuclear envelope-bridging complexes in homology-directed repair. *Mol Biol Cell* **25**, 2461–2471.

47 Dhingra N, Wei L and Zhao X (2019) Replication protein a (RPA) sumoylation positively influences the DNA damage checkpoint response in yeast. *J Biol Chem* **294**, 2690–2699.

48 Silva S, Altmannova V, Eckert-Boulet N, Kolesar P, Gallina I, Hang L, Chung I, Arneric M, Zhao X, Buron LD *et al.* (2016) SUMOylation of Rad52-Rad59 synergistically change the outcome of mitotic recombination. *DNA Repair (Amst)* **42**, 11–25.

49 Leland BA and King MC (2014) Using LacO arrays to monitor DNA double-strand break dynamics in live Schizosaccharomyces pombe cells. *Methods Mol Biol* **1176**, 127–141.

50 Taddei A and Gasser SM (2012) Structure and function in the budding yeast nucleus. *Genetics* **192**, 107–129.

51 Agmon N, Liefshitz B, Zimmer C, Fabre E and Kupiec M (2013) Effect of nuclear architecture on the efficiency of double-strand break repair. *Nat Cell Biol* **15**, 694–699.

52 Lee CS, Wang RW, Chang HH, Capurso D, Segal MR and Haber JE (2016) Chromosome position determines the success of double-strand break repair. *Proc Natl Acad Sci U S A* **113**, E146–E154.

53 Gehlen LR, Gasser SM and Dion V (2011) How broken DNA finds its template for repair: a computational approach. *Prog Theor Phys Supp* **191**, 20–29.

54 Wilson JH, Leung WY, Bosco G, Dieu D and Haber JE (1994) The frequency of gene targeting in yeast depends on the number of target copies. *Proc Natl Acad Sci USA* **91**, 177–181.

55 Batte A, Brocas C, Bordelet H, Hocher A, Ruault M, Adjiri A, Taddei A and Dubrana K (2017) Recombination at subtelomeres is regulated by physical distance, double-strand break resection and chromatin status. *EMBO J* **36**, 2609–2625.

56 Challa K, Schmid CD, Kitagawa S, Cheblal A, Iesmantavicius V, Seeber A, Amitai A, Seebacher J, Hauer MH, Shimada K *et al.* (2021) Damage-induced chromatome dynamics link ubiquitin ligase and proteasome recruitment to histone loss and efficient DNA repair. *Mol Cell* **81**, 811.e6–829.e6.

57 Albert S, Schaffer M, Beck F, Mosalaganti S, Asano S, Thomas HF, Plitzko JM, Beck M, Baumeister W and Engel BD (2017) Proteasomes tether to two distinct sites at the nuclear pore complex. *Proc Natl Acad Sci USA* **114**, 13726–13731.

58 Churikov D, Charifi F, Eckert-Boulet N, Silva S, Simon MN, Lisby M and Geli V (2016) SUMO-dependent Relocalization of eroded telomeres to nuclear pore complexes controls telomere recombination. *Cell Rep* **15**, 1242–1253.

59 Khadaroo B, Teixeira MT, Luciano P, Eckert-Boulet N, Germann SM, Simon MN, Gallina I, Abdallah P, Gilson E, Geli V *et al.* (2009) The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. *Nat Cell Biol* **11**, 980–987.

60 Mullen JR and Brill SJ (2008) Activation of the Slx5-Slx8 ubiquitin ligase by poly-small ubiquitin-like modifier conjugates. *J Biol Chem* **283**, 19912–19921.

61 Marcomini I, Shimada K, Delgoshaie N, Yamamoto I, Seeber A, Cheblal A, Horigome C, Naumann U and Gasser SM (2018) Asymmetric processing of DNA ends at a double-Strand break leads to unconstrained dynamics and ectopic translocation. *Cell Rep* **24**, 2614.e4–2628.e4.

62 Li SJ and Hochstrasser M (2003) The Ulp1 SUMO isopeptidase: distinct domains required for viability, nuclear envelope localization, and substrate specificity. *J Cell Biol* **160**, 1069–1081.

63 Zhao X, Wu CY and Blobel G (2004) Mlp-dependent anchorage and stabilization of a desumoylating enzyme is required to prevent clonal lethality. *J Cell Biol* **167**, 605–611.

64 Horigome C, Unozawa E, Ooki T and Kobayashi T (2019) Ribosomal RNA gene repeats associate with the nuclear pore complex for maintenance after DNA damage. *PLoS Genet* **15**, e1008103.

65 Wei L and Zhao X (2016) A new MCM modification cycle regulates DNA replication initiation. *Nat Struct Mol Biol* **23**, 209–216.

66 Meng X, Wei L, Peng XP and Zhao X (2019) Sumoylation of the DNA polymerase epsilon by the Smc5/6 complex contributes to DNA replication. *PLoS Genet* **15**, e1008426.

18733468, 2023, 22, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.14751 by Bcu Lausanne, Wiley Online Library on [09/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

67 Su XA, Dion V, Gasser SM and Freudenreich CH (2015) Regulation of recombination at yeast nuclear pores controls repair and triplet repeat stability. *Genes Dev* **29**, 1006–1017.
68 Geli V and Lisby M (2015) Recombinational DNA repair is regulated by compartmentalization of DNA lesions at the nuclear pore complex. *Bioessays* **37**, 1287–1292.
69 Seeber A and Gasser SM (2017) Chromatin organization and dynamics in double-strand break repair. *Curr Opin Genet Dev* **43**, 9–16.
70 Barker S, Weinfeld M and Murray D (2005) DNA-protein crosslinks: their induction, repair, and biological consequences. *Mutat Res* **589**, 111–135.
71 Fu YV, Yardimci H, Long DT, Ho TV, Guainazzi A, Bermudez VP, Hurwitz J, van Oijen A, Scharer OD and Walter JC (2011) Selective bypass of a lagging strand roadblock by the eukaryotic replicative DNA helicase. *Cell* **146**, 931–941.
72 Nakano T, Miyamoto-Matsubara M, Shoulkamy MI, Salem AM, Pack SP, Ishimi Y and Ide H (2013) Translocation and stability of replicative DNA helicases upon encountering DNA-protein cross-links. *J Biol Chem* **288**, 4649–4658.
73 Ide H, Nakano T, Salem AMH and Shoulkamy MI (2018) DNA-protein cross-links: formidable challenges to maintaining genome integrity. *DNA Repair (Amst)* **71**, 190–197.
74 Klages-Mundt NL and Li L (2017) Formation and repair of DNA-protein crosslink damage. *Sci China Life Sci* **60**, 1065–1076.
75 Pommier Y, Sun Y, Huang SN and Nitiss JL (2016) Roles of eukaryotic topoisomerases in transcription, replication and genomic stability. *Nat Rev Mol Cell Biol* **17**, 703–721.
76 Stingele J, Bellelli R and Boulton SJ (2017) Mechanisms of DNA-protein crosslink repair. *Nat Rev Mol Cell Biol* **18**, 563–573.
77 Staker BL, Hjerrild K, Feese MD, Behnke CA, Burgin AB Jr and Stewart L (2002) The mechanism of topoisomerase I poisoning by a camptothecin analog. *Proc Natl Acad Sci USA* **99**, 15387–15392.
78 Kawale AS and Povirk LF (2018) Tyrosyl-DNA phosphodiesterases: rescuing the genome from the risks of relaxation. *Nucleic Acids Res* **46**, 520–537.
79 Pommier Y, Huang SY, Gao R, Das BB, Murai J and Marchand C (2014) Tyrosyl-DNA-phosphodiesterases (TDP1 and TDP2). *DNA Repair (Amst)* **19**, 114–129.
80 Lopez-Mosqueda J, Maddi K, Prgomet S, Kalayil S, Marinovic-Terzic I, Terzic J and Dikic I (2016) SPRTN is a mammalian DNA-binding metalloprotease that resolves DNA-protein crosslinks. *Elife* **5**, e21491.
81 Reinking HK, Hofmann K and Stingele J (2020) Function and evolution of the DNA-protein crosslink proteases Wss1 and SPRTN. *DNA Repair (Amst)* **88**, 102822.
82 Stingele J, Bellelli R, Alte F, Hewitt G, Sarek G, Maslen SL, Tsutakawa SE, Borg A, Kjaer S, Tainer JA *et al.* (2016) Mechanism and regulation of DNA-protein crosslink repair by the DNA-dependent metalloprotease SPRTN. *Mol Cell* **64**, 688–703.
83 Stingele J, Schwarz MS, Bloemeke N, Wolf PG and Jentsch S (2014) A DNA-dependent protease involved in DNA-protein crosslink repair. *Cell* **158**, 327–338.
84 Vaz B, Popovic M, Newman JA, Fielden J, Aitkenhead H, Halder S, Singh AN, Vendrell I, Fischer R, Torrecilla I *et al.* (2016) Metalloprotease SPRTN/DVC1 orchestrates replication-coupled DNA-protein crosslink repair. *Mol Cell* **64**, 704–719.
85 Balakirev MY, Mullally JE, Favier A, Assard N, Sulpice E, Lindsey DF, Rulina AV, Gidrol X and Wilkinson KD (2015) Wss1 metalloprotease partners with Cdc48/Doa1 in processing genotoxic SUMO conjugates. *Elife* **4**, e06763.
86 Noireterre A, Serbyn N, Bagdiul I and Stutz F (2023) Ubx5-Cdc48 assists the protease Wss1 at DNA-protein crosslink sites in yeast. *EMBO J* **42**, e113609.
87 Kuhbacher U and Duxin JP (2020) How to fix DNA-protein crosslinks. *DNA Repair (Amst)* **94**, 102924.
88 Weickert P and Stingele J (2022) DNA-protein crosslinks and their resolution. *Annu Rev Biochem* **91**, 157–181.
89 Michel AH, Hatakeyama R, Kimmig P, Arter M, Peter M, Matos J, De Virgilio C and Kornmann B (2017) Functional mapping of yeast genomes by saturated transposition. *Elife* **6**, e23570.
90 Serbyn N, Noireterre A, Bagdiul I, Plank M, Michel AH, Loewith R, Kornmann B and Stutz F (2020) The aspartic protease Ddi1 contributes to DNA-protein crosslink repair in yeast. *Mol Cell* **77**, 1066.e9–1079.e9.
91 Yip MCJ, Bodnar NO and Rapoport TA (2020) Ddi1 is a ubiquitin-dependent protease. *Proc Natl Acad Sci USA* **117**, 7776–7781.
92 Nielsen I, Bentsen IB, Lisby M, Hansen S, Mundbjerg K, Andersen AH and Bjergbaek L (2009) A Flp-nick system to study repair of a single protein-bound nick *in vivo*. *Nat Methods* **6**, 753–757.
93 Serbyn N, Bagdiul I, Noireterre A, Michel AH, Suhandynata RT, Zhou H, Kornmann B and Stutz F (2021) SUMO orchestrates multiple alternative DNA-protein crosslink repair pathways. *Cell Rep* **37**, 110034.
94 Fielden J, Wiseman K, Torrecilla I, Li S, Hume S, Chiang SC, Ruggiano A, Narayan Singh A, Freire R, Hassanieh S *et al.* (2020) TEX264 coordinates p97- and SPRTN-mediated resolution of topoisomerase 1-DNA adducts. *Nat Commun* **11**, 1274.
95 Akhtar A and Gasser SM (2007) The nuclear envelope and transcriptional control. *Nat Rev Genet* **8**, 507–517.

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96 Bonnet A and Palancade B (2014) Regulation of mRNA trafficking by nuclear pore complexes. *Genes (Basel)* **5**, 767–791.
97 Casolari JM, Brown CR, Komili S, West J, Hieronymus H and Silver PA (2004) Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. *Cell* **117**, 427–439.
98 Taddei A, Van Houwe G, Hediger F, Kalck V, Cubizolles F, Schober H and Gasser SM (2006) Nuclear pore association confers optimal expression levels for an inducible yeast gene. *Nature* **441**, 774–778.
99 Dieppois G and Stutz F (2010) Connecting the transcription site to the nuclear pore: a multi-tether process that regulates gene expression. *J Cell Sci* **123**, 1989–1999.
100 Tutucci E and Stutz F (2011) Keeping mRNPs in check during assembly and nuclear export. *Nat Rev Mol Cell Biol* **12**, 377–384.
101 Sumner MC and Brickner J (2022) The nuclear pore complex as a transcription regulator. *Cold Spring Harb Perspect Biol* **14**, a039438.
102 Texari L, Dieppois G, Vinciguerra P, Contreras MP, Groner A, Letourneau A and Stutz F (2013) The nuclear pore regulates GAL1 gene transcription by controlling the localization of the SUMO protease Ulp1. *Mol Cell* **51**, 807–818.
103 Saik NO, Park N, Ptak C, Adames N, Aitchison JD and Wozniak RW (2020) Recruitment of an activated gene to the yeast nuclear pore complex requires Sumoylation. *Front Genet* **11**, 174.
104 Kurshakova MM, Krasnov AN, Kopytova DV, Shidlovskii YV, Nikolenko JV, Nabirochkina EN, Spehner D, Schultz P, Tora L and Georgieva SG (2007) SAGA and a novel drosophila export complex anchor efficient transcription and mRNA export to NPC. *EMBO J* **26**, 4956–4965.
105 Rohner S, Kalck V, Wang X, Ikegami K, Lieb JD, Gasser SM and Meister P (2013) Promoter- and RNA polymerase II-dependent hsp-16 gene association with nuclear pores in Caenorhabditis elegans. *J Cell Biol* **200**, 589–604.
106 Sterner DE, Nathan D, Reindle A, Johnson ES and Berger SL (2006) Sumoylation of the yeast Gcn5 protein. *Biochemistry* **45**, 1035–1042.
107 Andrulis ED, Zappulla DC, Ansari A, Perrod S, Laiosa CV, Gartenberg MR and Sternglanz R (2002) Esc1, a nuclear periphery protein required for Sir4-based plasmid anchoring and partitioning. *Mol Cell Biol* **22**, 8292–8301.
108 Hediger F, Neumann FR, Van Houwe G, Dubrana K and Gasser SM (2002) Live imaging of telomeres: yKu and sir proteins define redundant telomere-anchoring pathways in yeast. *Curr Biol* **12**, 2076–2089.
109 Taddei A and Gasser SM (2004) Multiple pathways for telomere tethering: functional implications of subnuclear position for heterochromatin formation. *Biochim Biophys Acta* **1677**, 120–128.
110 Ferreira HC, Luke B, Schober H, Kalck V, Lingner J and Gasser SM (2011) The PIAS homologue Siz2 regulates perinuclear telomere position and telomerase activity in budding yeast. *Nat Cell Biol* **13**, 867–874.
111 Schober H, Ferreira H, Kalck V, Gehlen LR and Gasser SM (2009) Yeast telomerase and the SUN domain protein Mps3 anchor telomeres and repress subtelomeric recombination. *Genes Dev* **23**, 928–938.
112 Ptak C, Saik NO, Premashankar A, Lapetina DL, Aitchison JD, Montpetit B and Wozniak RW (2021) Phosphorylation-dependent mitotic SUMOylation drives nuclear envelope-chromatin interactions. *J Cell Biol* **220**, e202103036.
113 Saik NO, Ptak C, Rehman S, Aitchison JD, Montpetit B and Wozniak RW (2023) SUMOylation at the inner nuclear membrane facilitates nuclear envelope biogenesis during mitosis. *J Cell Biol* **222**.
114 Lapetina DL, Ptak C, Roesner UK and Wozniak RW (2017) Yeast silencing factor Sir4 and a subset of nucleoporins form a complex distinct from nuclear pore complexes. *J Cell Biol* **216**, 3145–3159.
115 Taddei A, Hediger F, Neumann FR, Bauer C and Gasser SM (2004) Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. *EMBO J* **23**, 1301–1312.
116 Van de Vosse DW, Wan Y, Lapetina DL, Chen WM, Chiang JH, Aitchison JD and Wozniak RW (2013) A role for the nucleoporin Nup170p in chromatin structure and gene silencing. *Cell* **152**, 969–983.
117 Kueng S, Oppikofer M and Gasser SM (2013) SIR proteins and the assembly of silent chromatin in budding yeast. *Annu Rev Genet* **47**, 275–306.
118 Hediger F, Berthiau AS, van Houwe G, Gilson E and Gasser SM (2006) Subtelomeric factors antagonize telomere anchoring and Tel1-independent telomere length regulation. *EMBO J* **25**, 857–867.
119 Taddei A, Gartenberg MR, Neumann FR, Hediger F and Gasser SM (2005) Multiple pathways tether telomeres and silent chromatin at the nuclear periphery: functional implications for sir-mediated repression. *Novartis Found Symp* **264**, 140–156; discussion 156–165, 227–130.
120 Taddei A, Schober H and Gasser SM (2010) The budding yeast nucleus. *Cold Spring Harb Perspect Biol* **2**, a000612.
121 Ferreira HC, Towbin BD, Jegou T and Gasser SM (2013) The shelterin protein POT-1 anchors Caenorhabditis elegans telomeres through SUN-1 at the nuclear periphery. *J Cell Biol* **203**, 727–735.
122 Maison C, Bailly D, Quivy JP and Almouzni G (2016) The methyltransferase Suv39h1 links the SUMO pathway to HP1alpha marking at pericentric heterochromatin. *Nat Commun* **7**, 12224.

18733468, 2023, 22, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.14751 by Bcu Lausanne, Wiley Online Library on [09/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

123 Maison C, Romeo K, Bailly D, Dubarry M, Quivy JP and Almouzni G (2012) The SUMO protease SENP7 is a critical component to ensure HP1 enrichment at pericentric heterochromatin. *Nat Struct Mol Biol* **19**, 458–460.
124 Romeo K, Louault Y, Cantaloube S, Loiodice I, Almouzni G and Quivy JP (2015) The SENP7 SUMO-protease presents a module of two HP1 interaction motifs that locks HP1 protein at Pericentric heterochromatin. *Cell Rep* **10**, 771–782.
125 Moriuchi T, Kuroda M, Kusumoto F, Osumi T and Hirose F (2016) Lamin a reassembly at the end of mitosis is regulated by its SUMO-interacting motif. *Exp Cell Res* **342**, 83–94.
126 Aguilera P, Whalen J, Minguet C, Churikov D, Freudenreich C, Simon MN and Geli V (2020) The nuclear pore complex prevents sister chromatid recombination during replicative senescence. *Nat Commun* **11**, 160.
127 Lescasse R, Pobiega S, Callebaut I and Marcand S (2013) End-joining inhibition at telomeres requires the translocase and polySUMO-dependent ubiquitin ligase Uls1. *EMBO J* **32**, 805–815.
128 Bruhl J, Trautwein J, Schafer A, Linne U and Bouazoune K (2019) The DNA repair protein SHPRH is a nucleosome-stimulated ATPase and a nucleosome-E3 ubiquitin ligase. *Epigenetics Chromatin* **12**, 52.
129 Qu Y, Gharbi N, Yuan X, Olsen JR, Blicher P, Dalhus B, Brokstad KA, Lin B, Oyan AM, Zhang W *et al.* (2016) Axitinib blocks Wnt/beta-catenin signaling and directs asymmetric cell division in cancer. *Proc Natl Acad Sci USA* **113**, 9339–9344.
130 Unk I, Hajdu I, Blastyak A and Haracska L (2010) Role of yeast Rad5 and its human orthologs, HLTF and SHPRH in DNA damage tolerance. *DNA Repair (Amst)* **9**, 257–267.
131 Seeber A, Hegnauer AM, Hustedt N, Deshpande I, Poli J, Eglinger J, Pasero P, Gut H, Shinohara M, Hopfner KP *et al.* (2016) RPA mediates recruitment of MRX to forks and double-Strand breaks to hold sister chromatids together. *Mol Cell* **64**, 951–966.
132 Unal E, Heidinger-Pauli JM and Koshland D (2007) DNA double-strand breaks trigger genome-wide sister-chromatid cohesion through Eco1 (Ctf7). *Science* **317**, 245–248.
133 Chen YJ, Chuang YC, Chuang CN, Cheng YH, Chang CR, Leng CH and Wang TF (2016) S. Cerevisiae Mre11 recruits conjugated SUMO moieties to facilitate the assembly and function of the Mre11-Rad50-Xrs2 complex. *Nucleic Acids Res* **44**, 2199–2213.
134 Heckmann I, Kern MJ, Pfander B and Jentsch S (2019) A SUMO-dependent pathway controls elongating RNA polymerase II upon UV-induced damage. *Sci Rep* **9**, 17914.
135 Jalal D, Chalissery J and Hassan AH (2017) Genome maintenance in Saccharomyces cerevisiae: the role of SUMO and SUMO-targeted ubiquitin ligases. *Nucleic Acids Res* **45**, 2242–2261.
136 Hopfler M, Kern MJ, Straub T, Prytuliak R, Habermann BH, Pfander B and Jentsch S (2019) Slx5/Slx8-dependent ubiquitin hotspots on chromatin contribute to stress tolerance. *EMBO J* **38**, e100368.
137 Hauer MH and Gasser SM (2017) Chromatin and nucleosome dynamics in DNA damage and repair. *Genes Dev* **31**, 2204–2221.
138 Hauer MH, Seeber A, Singh V, Thierry R, Sack R, Amitai A, Kryzhanovska M, Eglinger J, Holcman D, Owen-Hughes T *et al.* (2017) Histone degradation in response to DNA damage enhances chromatin dynamics and recombination rates. *Nat Struct Mol Biol* **24**, 99–107.

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