Intricate SUMO-based control of the homologous recombination machinery

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The homologous recombination (HR) machinery plays multiple roles in genome maintenance. Best studied in the context of DNA double-stranded break (DSB) repair, recombination enzymes can cleave, pair, and unwind DNA molecules, and collaborate with regulatory proteins to execute multiple DNA processing steps before generating specific repair products. HR proteins also help to cope with problems arising from DNA replication, modulating impaired replication forks or filling DNA gaps. Given these important roles, it is not surprising that each HR step is subject to complex regulation to adjust repair efficiency and outcomes as well as to limit toxic intermediates. Recent studies have revealed intricate regulation of all steps of HR by the protein modifier SUMO, which has been increasingly recognized for its broad influence in nuclear functions. This review aims to connect established roles of SUMO with its newly identified effects on recombinational repair and stimulate further thought on many unanswered questions.

Homologous recombination is critical for several aspects of life, ranging from DNA repair and genome duplication to gamete production. Our understanding of HR pathways has benefited from a combination of assay systems. In cells, the generation of a defined DSB allows quantitative assessment of the status of the broken DNA molecules and the repair proteins at a temporal resolution, as well as determination of the genetic requirement for each step of repair (Haber 2016). Extensive biochemical analyses and more recently single molecule experiments have further defined the activities of HR enzymes and elucidated how they can collaborate in multiple HR steps. Several recent reviews have discussed these findings in detail (Symington et al. 2014; Heyer 2015; Ranjha et al. 2018); thus, we give only a brief overview here for each HR step to provide the context of SUMO-based regulation. As the HR machinery and its sumoylation are best examined in budding yeast, we use this system as an index for summarizing SUMO-based control. We also discuss additional regulation in mammalian cells and highlight their similarities and differences with those found in yeast. It is noteworthy that SUMO plays important roles in modulating protein recruitment to damaged chromatin and in other DNA break repair pathways. As these topics have been well covered in other reviews (Schwertman et al. 2016; Garvin and Morris 2017), they are not addressed here in order to maintain the focus on the regulation of core HR machinery.

Overview of the SUMO pathway and the effects of sumoylation

SUMO, a small protein of ~100 residues, is highly conserved among eukaryotes with several isoforms found in mammalian cells. SUMO can be conjugated to the lysine residues of target proteins via the action of SUMO E1, E2, and E3 enzymes (Fig. 1; Johnson 2004). The SUMO E1 helps to covalently link SUMO to the E2 enzyme's active site. In many cases, SUMO E2 can directly bind to the sumoylation consensus sequence ψ-K-X-E/D (ψ: hydrophobic residue) or its reverse sequence on the target proteins, permitting the transfer of SUMO to the lysine residue (Rodriguez et al. 2001; Sampson et al. 2001). However, in vivo sumoylation often requires SUMO E3s, which promote productive configurations for SUMO transfer by simultaneously binding the SUMO-charged E2 and the substrate (Werner et al. 2012; Streich and Lima 2016). Organisms examined so far contain a single SUMO E1 and E2 but multiple SUMO E3s to confer some levels of substrate specificity. However, substrate overlap is also seen for SUMO E3s. Such redundancy can increase the probability of sumoylation and thus the robustness of SUMO-based regulation. Earlier genetic studies have implicated sumoylation enzymes in the regulation of HR and genome stability from yeast to humans (Xhemalce et al. 2004; Zhao and Blobel 2005; Branzei et al. 2006; Galanty et al. 2009; Morris et al. 2009). Further examination of how sumoylation affects substrate properties has begun to shed light on the basis of SUMO-mediated regulation.

Figure 1. A simplified scheme for the SUMO pathway and consequences of sumoylation. Sumoylation of a substrate requires the sequential action of SUMO E1, E2, and E3 enzymes. The SUMO moiety (S) covalently linked to the substrates can modulate protein functions in different ways (see text for detail). Reversal of sumoylation is catalyzed by the desumoylases. The yeast SUMO pathway enzymes are indicated. In some contexts, a sumoylated substrate can be recognized by SUMO pathway accessory factors, as represented by the budding yeast proteins Uls1, Cdc48, and Slx5/8. These accessory proteins can regulate the fates of sumoylated proteins in distinct manners as detailed in the text.

Collective evidence suggests that the SUMO moiety can change substrate attributes in multiple ways, including altering its activities or interactions with other biomolecules (Flotho and Melchior 2013; Pichler et al. 2017). The diversity of SUMO's effects is described below in the context of each HR step. It is worthwhile to highlight a prevalent effect of sumoylation that is mediated by binding to SUMO interaction motifs (SIMs) composed of a hydrophobic core preceded or followed by negatively charged residues (Song et al. 2004; Hecker et al. 2006). The SUMO:SIM interaction is relatively weak, a feature that can actually be useful for modulating dynamic processes involving many protein hand-over events such as HR. Indeed, multiple HR factors contain SIMs, some of which have been shown to promote interactions with sumoylated proteins while others await further examination (detailed below). The SUMO:SIM interaction can also aid more stable association if additional binding interfaces are present. In some instances, stronger interactions are established when several SIMs from a protein bind to multiple SUMO moieties conjugated to the substrates or to SUMO itself (SUMO chains).

A family of multi-SIM containing proteins, STUbLs (SUMO targeted ubiquitin ligases), have a wide-ranging influence on HR by ubiquitinating sumoylated proteins or targeting the DNA bound by these proteins, such as unrepairable DNA breaks, damaged replication forks, or broken heterochromatin, to the nuclear periphery (Nie and Boddy 2016; Seeber and Gasser 2017). Another family of multi-SIM proteins, represented by the budding yeast Uls1 and fission yeast Rrp1/2, possess a SWI2/SNF2 ATPase domain and may be able to remove sumoylated proteins from chromatin via their potential translocase activities (Shah et al. 2010; Lescasse et al. 2013; Wei et al. 2017). Yet another way to extract sumoylated pro-

teins from chromatin or from protein association is mediated by the Cdc48 segregase (Torrecilla et al. 2017). In both budding and fission yeasts, the Cdc48 cofactor Ufd1 uses its SIM sequences to recognize sumoylated proteins, targeting the segregase to specific substrates for their extraction and sometimes degradation (Bergink et al. 2013; Kohler et al. 2013). Collectively, STUbLs, Uls1 family proteins, and the Cdc48 segregase provide multiple avenues to regulate the fates of the SUMO targets (Fig. 1).

Competing with these accessory factors are the SUMO-specific proteases or desumoylases that can cleave SUMO off these proteins or collapse SUMO chains (Fig. 1; Hickey et al. 2012). The distinct cellular localization of these desumoylases provides an important strategy to diversify their substrate repertoire. The multiple means to remove SUMO from proteins or degrade sumoylated proteins attest to the highly dynamic nature of cellular sumoylation, and the difficulty of detecting the low levels of sumoylated forms. In addition, the so-called "on-site" sumoylation, wherein DNA metabolism factors are only sumoylated once they have engaged with DNA for specific transactions, can also explain the low levels of sumoylated forms detected in vivo (Sarangi and Zhao 2015). It is important to comprehend how the SUMO forms of substrates can exert specific effects before their elimination by the factors described above. Although multiple challenging issues remain to be addressed, studies of the sumoylation of HR proteins have provided insights into this question as summarized below.

DNA end resection factors, their roles in sumoylation, and their regulation by SUMO

The canonical HR pathway that repairs DSBs can be roughly divided into three stages that are conserved from yeast to humans. In the first stage, the DNA end resection machinery generates 3′ single strand DNA (ssDNA) to be used for homology searching (Symington 2016). In budding yeast, DNA end resection involves the initial trimming of 5′ DNA ends by the MRX (Mre11–Rad50–Xrs2) nuclease in collaboration with Sae2, which is followed by extended DNA degradation by either the helicase-nuclease duo Sgs1 (with cofactor Top3-Rmi1) and Dna2, or the exonuclease Exo1 (Fig. 2; Symington 2016).

The resultant ssDNA is bound by the ssDNA-binding complex RPA (Rfa1–3) to prevent secondary structure formation or DNA degradation. In addition, the RPA-ssDNA filament provides a platform for recruiting the yeast SUMO E3 Siz2 that contributes to the sumoylation of RPA itself as well as the downstream HR proteins Rad52 and 59 (more below) (Chung and Zhao 2015). This recruitment requires the protein-binding module of the Rfa2 subunit and a region extended from the SAP domain of Siz2 (Chung and Zhao 2015). Since the RPA-ssDNA filament is a universal intermediate in DNA metabolic processes, it is tempting to speculate that the RPA–Siz2 interaction can also promote sumoylation in other DNA repair contexts and activate on-site sumoylation in other ssDNA
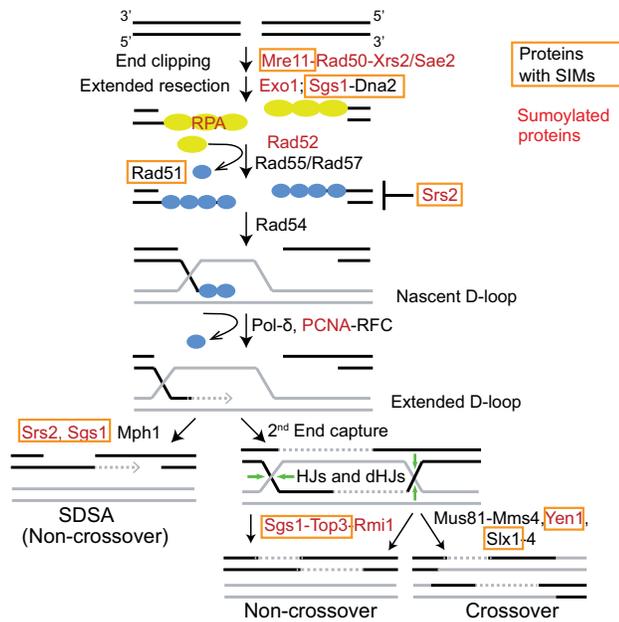
Figure 2. Summary of HR processes and HR proteins reported for sumoylation and interacting with SUMO. The figure depicts an overview of recombinational repair of DSBs with only the budding yeast proteins shown for simplicity. Recombinational repair is initiated by a two-step 5′ end resection process to generate 3′ end ssDNA, which can be coated by RPA. Several HR mediator proteins can then help to replace RPA with the Rad51 recombinase. With the assistance of Rad54, the Rad51-ssDNA filament can pair and invade donor DNA, leading to the formation of nascent D-loop structures. New DNA synthesis by Polδ with the help of its cofactor PCNA can extend the D-loop structures, which can be subsequently processed in two different ways. In the SDSA pathway, DNA helicases, such as Srs2, Sgs1, and Mph1, can remove ssDNA from donor DNA, leading to noncrossover outcomes. Alternatively, second end capture can lead to the formation of Holliday junction (HJ) structures. These structures can be processed by the dissolution enzymes (Sgs1–Top3–Rmi1) to generate exclusively noncrossover products or by the resolution nucleases (Mus81–Mms4, Yen1, and Slx1/4) to produce both noncrossover and crossover products. Note that the Srs2 helicase plays multiple roles during DSB repair, and only its role in disfavoring D-loop formation is depicted here for simplicity (see the text for details). Proteins reported to be sumoylated are in red and those reported to contain SIMs are boxed in orange.

regions. As SUMO enzymes are recruited to DSB sites in mammalian cells as well (Galanty et al. 2009; Morris et al. 2009), it will be interesting to define their recruitment mechanisms and the roles of RPA in this process.

The MRX complex is also required for Siz2-mediated sumoylation of RPA and Rad52 and Rad59 (Cremona et al. 2012; Psakhye and Jentsch 2012). As lack of Sae2 and Exo1 has similar effects on sumoylation as mutants of MRX, these end resection factors likely contribute to sumoylation via ssDNA generation that permits Siz2 recruitment (Cremona et al. 2012; Psakhye and Jentsch 2012; Chung and Zhao 2015). This theory does not exclude another proposal that MRX may have a direct role in recruiting the SUMO machinery, which is based on

yeast two-hybrid interactions of Mre11 with SUMO, SUMO E2, and Siz2 (Chen et al. 2016). Clarifying how Mre11 associates with these proteins and its direct involvement in their recruitment will provide further insight into the roles of MRX in sumoylation. Regardless, it is noteworthy that the requirement of RPA and MRX in sumoylation reminisces their roles in DNA damage checkpoint activation. However, DNA damage-induced sumoylation and checkpoint activation are largely separable, albeit with some degree of crosstalk (Cremona et al. 2012; Wu et al. 2014). For example, eliminating the main checkpoint kinase Mec1 in yeast does not reduce bulk sumoylation, but the sumoylation of the ATRIP checkpoint protein in human cells and RPA in yeast positively affects checkpoint function (Cremona et al. 2012; Wu et al. 2014; Dhingra et al. 2019).

Not only do DNA end resection factors affect sumoylation, they are also subject to SUMO-based regulation in yeast and mammalian cells. Sumoylation has an overall positive effect on end resection, though the underlying mechanisms are only partially understood. SUMO can increase Sae2 solubility, which may be reminiscent of an "antiglue" effect of sumoylation on the polyglutamine-containing protein ataxin-7 acting in chromatin remodeling and the aggregation-prone α-synuclein implicated in Parkinson's disease (Janer et al. 2010; Krumova et al. 2011; Sarangi et al. 2015). SUMO exerts a stronger functional influence on the Sae2 human homolog, CtIP, by yet to be identified mechanisms (Soria-Bretones et al. 2017). In addition, sumoylation is suggested to promote the EXO1 protein stability in mammalian cells via direct or indirect means (Bologna et al. 2015). The roles of sumoylation of the yeast MRX or its mammalian counterpart MRN (MRE11–RAD50–NBS1) are also unclear, but enhancement of MRN sumoylation by an adenovirus SUMO E3 suggests a possible effect on the host–virus interaction (Cremona et al. 2012; Psakhye and Jentsch 2012; Sohn and Hearing 2012). Further studies on how SUMO influences the activities of these nucleases can also shed light on end resection in other cellular contexts, such as at stalled or collapsed replication forks, where DNA degradation needs to be tightly regulated.

SUMO exerts complex control of Rad51 filament formation and functions

The second stage of HR repair involves homology search and DNA synthesis to generate recombination intermediates (RIs) in the form of joint DNA molecules (Symington et al. 2014; Heyer 2015; Ranjha et al. 2018). First, the Rad51 recombinase replaces RPA on ssDNA, forming a presynaptic filament capable of homology search and pairing with donor DNA. Successful pairing leads to the formation of a nascent D-loop structure, which can be extended by DNA polymerase delta, resulting in the formation of an extended D-loop structure (Fig. 2). Loading Rad51 onto ssDNA requires mediator proteins, while generating D-loop structures (D-loops) depends on Rad54 and its homologs. In yeast, the most essential mediator is Rad52, with Rad55 and Rad57 also being critical in the

repair of DSBs and the four-subunit Shu complex mostly required in replication-associated HR (Krejci et al. 2012).

Among HR proteins that catalyze the formation of D-loops, only Rad52 is known to be sumoylated (Sacher et al. 2006; Cremona et al. 2012; Psakhye and Jentsch 2012) and several models have been put forward to explain SUMO's effects on Rad52 functions. It was initially suggested that Rad52 sumoylation promotes its interaction with Rad51, favoring RI generation (Sacher et al. 2006). This notion is supported by genetic data that Rad52 sumoylation loss rescues the DNA damage sensitivity of several mutants that are sensitive to RI accumulation, such as cells lacking both Sgs1 and the Srs2 helicases that can remove Rad51 from DNA (Sacher et al. 2006). Later on, sumoylation of Rad52 was found to recruit the Cdc48 segregase to disfavor Rad52 interaction with Rad51 and promotes its degradation (Bergink et al. 2013). Other studies found that Rad52 sumoylation promotes its interaction with Rad59, a Rad52 paralog that catalyzes Rad51-independent repair, thus fostering Rad59-mediated repair at the expense of Rad51-mediated repair (Altman-nova et al. 2010; Silva et al. 2016). Yet another model posits that sumoylation of Rad52 can diminish its ability to counter Srs2-mediated removal of Rad51 from ssDNA, as SUMO-fused Rad52 suppresses *srs2Δ* genotoxic sensitivity without affecting overall HR repair (Esta et al. 2013). Moreover, locus-specific effects of Rad52 sumoylation have also been shown wherein it can disfavor recombination foci formation in rDNA and centromeric regions (Torres-Rosell et al. 2007; Yong-Gonzales et al. 2012). A challenge now is to formulate a unified model for the roles of Rad52 sumoylation. For example, can the pro- and anti-Rad51 effects mediated by Rad52 sumoylation occur at different repair or chromatin contexts, and can Cdc48-dependent extraction take place at a later stage of repair after sumoylated Rad52 carries out its mediator or Rad59-mediated functions? Addressing these questions will be critical for achieving a better understanding of how sumoylation of a single mediator protein can regulate D-loop formation, repair pathway choices, and limit toxic recombination intermediates.

An antagonist of the HR mediator proteins, the Srs2 helicase, can both bind to SUMO and be sumoylated. Srs2 contains a C-terminal SIM best known for collaborating with a nearby PIM (PCNA interacting motif) to bind to sumoylated PCNA (Papouli et al. 2005; Pfander et al. 2005). This interaction was initially shown to recruit Srs2 to stalled replication forks where it can remove Rad51 from DNA (Papouli et al. 2005; Pfander et al. 2005). Consequently, Srs2 can disfavor the use of canonical HR as a means to restart replication, while favoring the Rad6/18-mediated DNA tolerance pathway (Hoege et al. 2002; Stelter and Ulrich 2003). However, the story seems to be more nuanced as the Srs2 interaction with sumoylated PCNA is not indiscriminately anti-HR but in fact favors the SDSA (synthesis dependent strand annealing) branch of recombinational repair. SDSA entails the displacement of the invaded ssDNA from extended D-loops and pairing it with the other 3′ ssDNA tail to allow subsequent gap synthesis and repair completion, generating ex-

clusively noncrossover products (Symington et al. 2014; Heyer 2015; Ranjha et al. 2018). Alternatively, ligation of the DNA end from the D-loop with the other broken DNA end would form Holliday junction structures, the cleavage of which can lead to either crossover and noncrossover products (Fig. 2; Symington et al. 2014; Heyer 2015; Ranjha et al. 2018). As crossovers cause genetic changes of surrounding regions and lead to loss-of-heterozygosity in diploid cells, SDSA is a preferred pathway in mitotic cells.

A role for Srs2 interaction with sumoylated PCNA in SDSA is supported by both genetic and biochemical data. Mutants disrupting this interaction exhibit increased rates of crossover and loss of heterozygosity (Robert et al. 2006; Le Breton et al. 2008; Miura et al. 2013). Biochemically, while the Srs2 helicase activity can displace the invaded ssDNA from nascent or extended D-loops, sumoylated PCNA confers a bias towards the latter (Liu et al. 2017). Srs2 binding to sumoylated PCNA was also shown to prevent DNA polymerase delta binding to PCNA, disfavoring D-loop extension (Burkovics et al. 2013). These combined effects may explain the ultimate result that Srs2 interaction with sumoylated PCNA reduces crossover levels. An Srs2-like protein in mammals, PARI, also interacts with sumoylated PCNA, disfavors Rad51 foci formation, and HR in cells (Moldovan et al. 2012). Unlike Srs2, PARI has no classic ATPase/helicase domain and does not display helicase activity; thus, its Rad51-antagonistic effect might stem from interacting with a motor protein to displace Rad51 on ssDNA or its stimulation of the Rad51 ATPase activity that can help this disassociation (Moldovan et al. 2012).

Srs2 is also sumoylated and eliminating this modification specifically impairs HR in the rDNA region, suggesting a unique context for Srs2 sumoylation to exert a detectable effect (Kolesar et al. 2012, 2016). Intriguingly, Srs2 sumoylation is toxic in the absence of CDK-mediated phosphorylation of Srs2 that is thought to promote SDSA (Saponaro et al. 2010). Whether this toxicity is related to a role for Srs2 sumoylation in rDNA recombination will be interesting to explore. More recently, Srs2 sumoylation has been linked to the regulation of its protein levels. The STUbL enzyme Slx5/8 interacts with Srs2 and can promote its degradation (Urulangodi et al. 2015). This regulation at replication forks involves another Srs2 interactor, Esc2, which associates with replication forks in vivo (Urulangodi et al. 2015). Esc2 contains two SUMO-like domains (SLD1/2) that foster its interaction with Srs2 in a manner dependent on Srs2's SIM (Urulangodi et al. 2015). The proposed model is that the two Srs2 interactors, Esc2 and Slx5/8, collaborate to reduce Srs2 levels at stalled replication forks (Urulangodi et al. 2015). This may provide a way to curb Srs2-mediated inhibition of Rad51 in situations when HR is needed to rescue replication defects. Adding to this picture, Uls1, which also interacts with Slx5/8, was recently shown to associate with Srs2 and restrain its levels as well (Kramarz et al. 2017). It will be interesting to gain a deeper understanding of the interplay between Uls1, Slx5/8, and Esc2 in Srs2

control, such as whether they act sequentially or at distinct DNA damage sites.

Yeast and mammalian Rad51 harbor a conserved SIM, mutants of which can reduce Rad51 accretion at DNA damage sites in human cells (Bergink et al. 2013; Shima et al. 2013). It is unclear how the Rad51-SIM exerts this effect, but an obvious possibility to test is whether it binds to other sumoylated HR proteins. In human cells, the UAF1 protein containing two SLD domains can bind to a Rad51 interactor, Rad51-AP1, in a manner depending on the SIMs of Rad51-AP1 (Liang et al. 2016). This interaction fosters Rad51-mediated pairing of ssDNA with donor dsDNA in vitro and enhances HR in vivo (Liang et al. 2016). Yet another way to promote Rad51 filament formation could involve the sumoylation of human RPA (Dou et al. 2010). In summary, current studies have shown multiple layers of SUMO-based regulation of RI formation and HR pathway choices. Many questions, as exemplified above, remain to be addressed to generate unified models of how these layers of regulation can achieve specific effects in different contexts, and whether the findings in yeast are conserved in human cells.

Sumoylation collaborates with phosphorylation to regulate HJ removal enzymes

During the last stage of HR repair, joint DNA molecules produced during the early steps, including HJs and D-loop structures, are processed to yield linear DNA products. Multiple enzymes with RI processing activity can generate different repair outcomes and are activated at different cell cycle stages (West et al. 2015). Dissolution of double HJs (dHJs) by the STR complex or its mammalian counterpart (BLM–TopIIIα–RMI) produces only noncrossover products; thus, it is favored in mitotic cells and is likely the main HJ removal activity in S phase. In addition, STR also dissolves D-loops, providing another means to remove RIs (Fasching et al. 2015; Piazza et al. 2019). In G2/M phase, the Mus81–Mms4 (human MUS81–EME1) structure-selective nuclease, which can cleave multiple types of RIs, is activated by CDK and Polo-like kinase, while the Yen1 (human GEN1) resolvase targeting single HJs is turned on only in anaphase by Cdc14-mediated dephosphorylation (Wild and Matos 2016). Both types of nucleases produce crossover and noncrossover products (Fig. 2). Thus, limiting their activities to the latter part of the cell cycle gives STR/BTR more time to generate noncrossover products, but still allows the removal of any remaining RIs before cell division. Another structure-selective DNA nuclease, the Slx1–Slx4 complex, also contributes to RI removal. The mammalian SLX4 harbors additional domains for binding to MUS81-EME1 and other proteins, gaining additional functions and regulation, which has been nicely summarized in a recent review (Guervilly and Gaillard 2018).

Recent studies have provided evidence that the aforementioned RI processing enzymes are subject to SUMO-based regulation. Bulk STR sumoylation requires upstream HR factors that produce RIs, suggesting on-site sumoylation for this complex (Bonner et al. 2016; Bermu-

dez-Lopez et al. 2016). Sumoylation of STR can then engender intersubunit interaction and its accrual at DNA damage sites, thus fostering RI removal (Bonner et al. 2016; Bermudez-Lopez et al. 2016). Another study highlights the requirement of Sgs1 sumoylation at telomeres that may also mediate its role in RI removal (Lu et al. 2010). STR sumoylation partly requires the Mms21 SUMO E3 subunit of the Smc5/6 complex, explaining some shared phenotypes of Smc5/6 and STR mutants, such as RI accumulation (Bonner et al. 2016; Bermudez-Lopez et al. 2016). Given that both Mms21 and STR also influence other processes such as replication fork regulation, it is possible that Mms21-mediated STR sumoylation may have roles beyond RI dissolution. Indeed, human BLM sumoylation, which also heavily relies on the human MMS21 homolog, appears to regulate collapsed replication forks. Sumoylation of BLM aids in its targeting to collapsed replication forks where it can both disfavor the accumulation of unproductive RIs and promote proper RAD51 functions (Ouyang et al. 2013; Pond et al. 2019). The underlying mechanisms for these two different effects remain to be elucidated.

Yen1 is also sumoylated and this modification was suggested to foster its interaction with Uls1 (Bauer et al. 2019). Genetic data support a positive influence of Uls1 on Yen1 function, which may explain why *uls1Δ* sensitizes *rad54/rdh54* mutants (Shah et al. 2010; Bauer et al. 2019). The story gets more complicated as Yen1 was also found to be ubiquitinated by Slx5/8 (Talhaoui et al. 2018). Given that both Slx5/8 and Uls1 can remove sumoylated proteins, it will be interesting to test whether they regulate Yen1 in this manner and how such a potential effect may influence HJ removal. In mammalian cells, both subunits of the MUS81-EME1 nuclease have been reported to be sumoylated, and MUS81 sumoylation is suggested to prevent chromosomal misalignment in mitosis, whereas the roles of EME1 sumoylation and whether the S phase-specific EME2 subunit is sumoylated remain to be determined (Xiao et al. 2015; Hu et al. 2017).

Mammalian SLX4 contains three SIM sequences that help to target SLX4 to DNA damage sites, telomeric regions, and PML bodies (Gonzalez-Prieto et al. 2015; Guervilly et al. 2015; Ouyang et al. 2015). The SLX4 SIMs could achieve this effect by fostering interaction with multiple partner proteins known to be sumoylated, such as RPA, MRN, and the telomere protein TRF2 (Ouyang et al. 2015). Interestingly, the SIMs of SLX4 were also found to promote the sumoylation of SLX4 and one of its binding partners XPF, suggesting the possibility that SLX4 can act as a SUMO E3 or an E3 cofactor (Guervilly et al. 2015). In support of this, SLX4 specifically binds to SUMO-charged E2 but not free E2 or SUMO (Guervilly et al. 2015). As SLX4 forms megacomplexes with additional nucleases and structural proteins involved in HJ removal, telomere metabolism, and replication fork regulation, it will be interesting to address whether SLX4 could broadly affect the sumoylation of its partner proteins, and whether the SIMs of SLX4 or its own sumoylation can regulate the formation of various SLX4-containing complexes and their functions. In yeast, the SUMO-binding

function is shifted from Slx4 to Slx1 and the Slx1:SUMO interaction favors binding to the Saw1 scaffold protein involved in strand annealing pathway and the repair of UV lesions (Sarangi et al. 2014). These observations both suggest a conserved role for SUMO in the Slx1–Slx4-mediated processes and the acquisition of more sophisticated SUMO-based regulation of the human SLX4 interactome.

Outlooks

The progress in the past decade or so has revealed how extensive the involvement of SUMO is in the regulation of HR factors and pathways. Despite the difficulty of detecting low levels of sumoylated proteins, the SUMO forms of many HR factors have been demonstrated and their sumoylation mutants have been examined. These studies have suggested different effects of sumoylation in the regulation of HR protein functions as summarized above. Several HR factors also contain SIM sequences that can recruit the SUMO pathway accessory factors, such as STUbLs, the Uls1 family proteins, and the segregase complex. However, as we uncover more details about SUMO-based regulation of HR, new questions arise, some of which are summarized above. For example, how can SUMO exert multiple distinct effects on the same substrate, how do the SUMO pathway accessory factors compete with desumoylases, what determines whether a sumoylated protein affects HR functions or be extracted or removed from DNA. Answering these questions requires both cellular studies employing sumoylation defective mutants and in vitro analyses of the behaviors of sumoylated proteins and their effects on specific HR reactions. In addition, new avenues of investigation, such as more mechanistic studies in mammalian cells, and examining the possible involvement of sumoylation in forming membrane-less repair centers in the nucleus, will continue to broaden our understanding of the roles of sumoylation in genome maintenance.

Acknowledgments

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