



Published in final edited form as:

Cell Rep. 2014 October 9; 9(1): 143–152. doi:10.1016/j.celrep.2014.08.054.

A Versatile Scaffold Contributes to Damage Survival via Sumoylation and Nuclease Interactions

Prabha Sarangi^{1,2,9}, Veronika Altmannova^{3,9}, Cory Holland^{4,11}, Zdenka Bartosova³, Fanfan Hao¹, Dorothea Anrather⁵, Gustav Ammerer⁵, Sang Eun Lee^{4,6}, Lumir Krejci^{3,7,8,10,*}, and Xiaolan Zhao^{1,2,10,*}

¹Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

²Programs in Biochemistry, Cell, and Molecular Biology, Weill Cornell Graduate School of Medical Sciences, New York, NY 10065, USA

³Department of Biology, Masaryk University, Brno 62500, Czech Republic

⁴Department of Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

⁵Department of Biochemistry and Cell Biology, Max F. Perutz Laboratories, University of Vienna, Vienna 1030, Austria

⁶Division of Radiation Biology, Department of Radiation Oncology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

⁷National Centre for Biomolecular Research, Masaryk University, Brno 62500, Czech Republic

⁸International Clinical Research Center, St. Anne's University Hospital in Brno, Brno 60200, Czech Republic

SUMMARY

DNA repair scaffolds mediate specific DNA and protein interactions in order to assist repair enzymes in recognizing and removing damaged sequences. Many scaffold proteins are dedicated to repairing a particular type of lesion. Here, we show that the budding yeast Saw1 scaffold is more versatile. It helps cells cope with base lesions and protein-DNA adducts through its known function of recruiting the Rad1-Rad10 nuclease to DNA. In addition, it promotes UV survival via a mechanism mediated by its sumoylation. Saw1 sumoylation favors its interaction with another

©2014 The Authors

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

*Correspondence: lkrejci@chemi.muni.cz (L.K.), zhaox1@mskcc.org (X.Z.).

⁹Co-first author

¹⁰Co-senior author

¹¹Present address: Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

AUTHOR CONTRIBUTIONS

P.S., V.A., L.K., and X.Z. conceived and designed the experiments, P.S., V.A., Z.B., and C.H. performed the experiments, F.H. generated some reagents, D.A. and G.A. performed the mass spectrometry experiments, P.S. and X.Z. analyzed the in vivo data, C.H. and S.E.L. analyzed the SSA results, V.A. and L.K. analyzed the in vitro data, P.S. and X.Z. wrote the paper, and V.A., C.H., S.E.L., and L.K. commented on the manuscript.

nuclease Slx1-Slx4, and this SUMO-mediated role is genetically separable from two main UV lesion repair processes. These effects of Saw1 and its sumoylation suggest that Saw1 is a multifunctional scaffold that can facilitate diverse types of DNA repair through its modification and nuclease interactions.

INTRODUCTION

Timely repair of the large number of DNA lesions occurring in the genome is critical to prevent mutations and other alterations of the genetic information. This task requires collaborations between individual DNA repair enzymes, as well as with scaffold proteins that aid some of these enzymes. In particular, DNA nucleases that remove damaged sequences from the genome often carry out their functions in conjunction with scaffold proteins (e.g., Guzder et al., 2006; Hammel et al., 2011; Prolla et al., 1994; Vidal et al., 2001).

Most repair scaffolds are thought to assist a particular repair process (Guzder et al., 2006; Hammel et al., 2011; Prolla et al., 1994; Vidal et al., 2001). The budding yeast scaffold protein Saw1 was recently shown to support single-strand annealing (SSA) repair of double-strand breaks (DSBs) (Li et al., 2008, 2013). SSA entails the annealing of resected DNA at repeat sequences adjacent to the break, the subsequent removal of nonhomologous flaps, and final ligation (Fishman-Lobell et al., 1992; reviewed in Heyer et al., 2010; Krogh and Symington, 2004). In SSA, Saw1 recruits the Rad1-Rad10 nuclease to the break sites for flap removal (Li et al., 2008, 2013). This recruitment requires the coordinated interactions of Saw1 with the nuclease, the flap DNA, and upstream SSA factors (Li et al., 2008, 2013). SSA is considered error-prone repair as it leads to deletions or translocations (Fishman-Lobell et al., 1992; Heyer et al., 2010; Krogh and Symington, 2004).

Although Saw1 is thought to be an SSA-specific scaffold, Rad1-Rad10 is involved in processes that repair other types of DNA lesions (Figure 1A). These include the repair of UV lesions via the nucleotide excision repair (NER) pathway (reviewed in Schärer, 2013), as well as backup repair of base lesions and protein-DNA adducts (Guillet and Boiteux, 2002; Vance and Wilson, 2002). Compared with error-prone SSA repair, these processes contribute to cellular survival in specific genotoxic environments. It has not been explored whether Saw1 can aid Rad1-Rad10 in these repair contexts, nor is it known if Saw1 has Rad1-independent roles in DNA repair.

Here, we show that Saw1 promotes survival in different genotoxic environments that generate base lesions, protein-DNA adducts, and UV lesions. Saw1 interactions with Rad1 and DNA flaps are required in the first two situations, suggesting that Saw1 assists Rad1-Rad10 in a broader range of DNA damage contexts than previously appreciated. In contrast, these known functions of Saw1 are not critical under UV condition, indicating that Saw1 also has Rad1-independent roles in specific lesion contexts. To elucidate this previously unknown aspect of Saw1's roles, we examined whether it is enabled by alteration of Saw1 function through protein modification. The only known modification of Saw1 is sumoylation, as reported by two recent proteomic screens (Cremona et al., 2012; Psakhye and Jentsch, 2012). We found that this modification is critical for Saw1-mediated UV

resistance partly due to collaboration with another DNA nuclease, Slx1-Slx4. Our findings highlight the versatility of the Saw1 nuclease scaffold in multiple damage contexts via collaborations with different repair factors and also provide an example whereby sumoylation of a repair scaffold differently regulates its functions.

RESULTS

Saw1-Mediated UV Resistance Is Separable from Its SSA Function

To understand if Saw1 has broader effects in repairing different types of DNA lesions beyond its known SSA function, we examined how cells lacking Saw1 cope with several DNA damaging agents. We first examined UV treatment, as the Saw1 binding partner, the Rad1-Rad10 nuclease, is critical for UV repair via the NER pathway (reviewed in Schärer, 2013; Figure 1A). We found that *saw1* cells exhibited increased UV sensitivity compared to wild-type cells (Figure 1B; see Table 1 for strain list). Because this sensitivity was less severe than that of *rad1* cells (Figure 1B), Saw1 is not the main Rad1 recruitment factor during UV repair, a notion consistent with the NER protein Rad14 being mainly responsible for Rad1 recruitment to UV lesions (Guzder et al., 2006).

Next, we asked whether the newly found UV sensitivity of *saw1* is attributable to defective SSA. To this end, we performed epistasis analysis with mutants lacking Rad59, a protein essential for SSA (Bai and Symington, 1996) (Figure 1A). We found that *saw1 rad59* cells were more UV sensitive than *rad59* cells (Figure 1C), indicating that the Saw1 contribution to UV resistance extends beyond SSA.

Saw1 Promotes Survival in Other Damage Conditions Independently of SSA

Next, we examined if *saw1* cells exhibit a phenotype indicative of defects in the repair of other types of DNA damage in which Rad1-Rad10 plays backup roles (Figure 1A). In the absence of base excision repair that requires the endonucleases Apn1 and Apn2, Rad1 becomes essential for cell growth (Boiteux and Guillet, 2004; Guillet and Boiteux, 2002). We found that *saw1* also showed the same genetic interaction with *apn1 apn2* as does *rad1*. This finding is consistent with idea that Saw1 is required for the backup repair of base lesions (Figure 1D). This function of Saw1 is separable from SSA, because *rad59* did not show similar synthetic lethality (Figure 1D).

The Rad1-Rad10 nuclease also acts in the backup repair of DNA linked to the topoisomerase Top1 (Figure 1A) (Vance and Wilson, 2002). Top1-DNA adducts are stabilized by camptothecin (CPT) and are primarily removed by the phosphodiesterase Tdp1 (Pouliot et al., 1999). In the absence of Tdp1, repair of Top1-DNA adducts by Rad1-Rad10 becomes critical, because *tdp1 rad1* cells are inviable on CPT-containing media (Vance and Wilson, 2002). We found that *tdp1 saw1* cells were also inviable when treated with CPT (Figure 1E), suggesting that Saw1 also contributes to Top1-DNA adduct situations. Again, this function of Saw1 is unrelated to SSA, because *rad59* does not sensitize *tdp1* cells (Vance and Wilson, 2002).

Taken together, the genetic evidence supports SSA-independent roles for Saw1 in survival under different DNA damage conditions. Next, we aimed to understand how a scaffold

protein performs these multiple tasks by examining whether posttranslational modification contributes to its diverse functions.

Saw1 Sumoylation Increases upon DNA Damage Treatment

Saw1 was found to be sumoylated in recent proteomic screens (Cremona et al., 2012; Psakhye and Jentsch, 2012). Consistent with these reports, a single sumoylated form of Saw1 from immunopurified samples was detected by western blotting using antibodies against SUMO or the TAP tag fused to the protein (Figure 2A). We note that as the F_c region of the SUMO antibody interacts with the Protein A part of TAP tag, it detects the unmodified protein, but more strongly so for the sumoylated form due to additional high affinity for SUMO (Cremona et al., 2012). Saw1 sumoylation was also detected in vitro in the presence of SUMO, sumoylation E1 and E2 enzymes, and ATP (Figure 2B, lane 2) (Altmannova et al., 2010).

To determine the SUMO E3s responsible for Saw1 sumoylation, we examined its modification levels in cells lacking function of the three mitotic E3s, namely, Siz1, Siz2, and Mms21 (Johnson and Gupta, 2001; Takahashi et al., 2001; Zhao and Blobel, 2005). Saw1 sumoylation was reduced in *siz1 siz2A* and *siz1 mms21* double mutants, but not in *siz2 mms21* or single E3 mutants in vivo (Figure 2A). In vitro, both Siz1 and Siz2 stimulated Saw1 sumoylation (Figure 2B, lanes 3 and 4). Thus, more than one SUMO ligase contributes to Saw1 sumoylation, making Saw1 yet another redundant E3 substrate (reviewed in Ulrich, 2009).

Because our findings suggest that Saw1 contributes to survival in the presence of multiple types of lesions, we examined Saw1 sumoylation under these DNA damage conditions. Saw1 sumoylation was greatly enhanced by treatment with UV, methylmethane sulfonate (MMS) that generates base lesions, and to a smaller extent by CPT (Figure 2C). This is in line with a role for Saw1 sumoylation in the repair of these lesions.

Saw1 Sumoylation Occurs at a Lysine outside Its Rad1- and Flap-Binding Motifs

To examine whether and how sumoylation affects the different functions of Saw1, we first mapped its sumoylation site. To this end, the sumoylated form of recombinant Saw1 was subjected to mass spectrometry analysis. This analysis identified lysine K221 as a candidate sumoylation site (Figure S1). Replacing this lysine with arginine at the endogenous locus eliminated Saw1 sumoylation in vivo (Figure 2D), confirming that K221 is the SUMO acceptor site in vivo.

Saw1 is a small protein with only two motifs identified thus far: a six amino acid Rad1-binding motif at the N terminus (referred to as RBD), and another six amino acid motif at the C terminus that is required for 3' flap binding in vitro (referred to as FBD) (Figure 3A) (Li et al., 2008,2013). Both motifs are absolutely required for Rad1 recruitment to 3' flaps in

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2014.08.054>.

SSA, and thus SSA repair (Li et al., 2008, 2013). Lysine 221 lies outside both motifs and is conserved among homologs in yeast species (Figure 3A; SGD database).

Saw1 -Mediated UV Resistance, but Not SSA, Relies on Its Sumoylation

We examined the phenotype of *saw1-K221R* and compared it with those of *saw1* null or mutants lacking either the Rad1 binding (*saw1-RBD*) or the flap binding (*saw1-FBD*) motifs. First, SSA efficiency was examined using an assay where the HO endonuclease-induced DSB is flanked by direct repeats (Li et al., 2008). Repair of this DSB is primarily mediated by SSA and can be scored by counting the colonies that survive DSB induction. *saw1*, *-RBD*, and *-FBD* mutants that cannot recruit Rad1 to 3' flaps show very poor survival and hence low SSA repair levels (Li et al., 2013). However, colony number for *saw1-K221R* cells was similar to that of wild-type (Figure 3B), suggesting that sumoylation of Saw1 is not required for SSA.

Next, we tested UV resistance. Figure 3C shows that *saw1-K221R* exhibited UV sensitivity similarly to *saw1*. This is in striking contrast to the SSA results and suggests that Saw1 sumoylation is required for its role in UV condition. As in the case of *saw1*, *saw1-K221R* sensitized *rad59* to UV (Figure 3D), further supporting the notion that *saw1-K221R* UV sensitivity is not due to an SSA defect.

Different from *saw1-K221R*, *saw1-FBD* showed only slight UV sensitivity, suggesting that flap binding is largely dispensable for UV resistance (Figure 3C). The UV sensitivity of *saw1-RBD* was in between that of *saw1-FBD* and *saw1-K221R* or null (Figure 3C), suggesting that the Saw1 contribution in the UV situation is only partly via assistance of Rad1-Rad10.

Saw1-Mediated Survival in Other Lesion Contexts Relies on Its Rad1 and DNA Flap Binding

We examined *saw1* mutants for phenotype indicative of defects in base lesion and CPT repair. Like *saw1*, *saw1-RBD*, and *-FBD* were synthetically lethal with *apn1 apn2*, and strongly sensitized *tdp1* to CPT (Figures 1D, 1E, 3E, and 3F). Thus, Saw1 interactions with Rad1 and 3' flap DNA are important for survival in the presence of base lesions and Top1-DNA adducts in these genetic backgrounds. Different from *saw1-RBD* and *-FBD*, *saw1-K221R apn1 apn2* cells were viable but exhibited slower growth and stronger MMS sensitivity than *apn1 apn2* (Figures 3E and 3G), and only moderate sensitization of *tdp1* cells to CPT (Figure 3F). These results suggest that Saw1 sumoylation only moderately promotes survival in the presence of base lesions and protein-DNA adducts.

Taken together, our genetic analyses suggest that the three attributes of Saw1, namely, Rad1 interaction, flap binding, and sumoylation, contribute to different extents in coping with different lesions (Figure 3H). In the UV case, Saw1 sumoylation is critical, whereas Rad1 and flap binding are less important. The reverse is true for MMS and CPT situations, as in SSA repair. Our data suggest that whereas Saw1 contributes to the latter three situations via the known mechanism of Rad1-Rad10 recruitment, its sumoylation affects the UV situation

largely independently of this mechanism. Next, we focused our efforts on understanding how Saw1 sumoylation promotes UV survival.

Saw1 Sumoylation Does Not Affect Protein Level, DNA Binding, or Rad1 Interaction

We first assessed protein levels of Saw1 in untreated and UV-treated cells and detected no difference between wild-type and *saw1-K221R* cells (Figures 4A and ^{S2A}), indicating that sumoylation of Saw1 does not affect bulk protein levels. Next, we examined how Saw1 sumoylation affects its DNA binding. Saw1 is a structure-specific DNA binding protein with affinity for branched DNA structures such as Y-forms (Li et al., 2013). We found that recombinant Saw1-K221R protein exhibited similar binding to Y-form DNA as its wild-type counterpart (Figure 4B). In addition, SUMO-Saw1 obtained by subjecting the protein to in vitro sumoylation that yielded ~40% modified protein as shown in Figure 2B (lane 3) showed no difference in binding affinity for Y-form DNA when compared with equal amounts of unmodified protein (Figure 4C). We also found that the Saw1 -K221R mutant was proficient for Rad1 interaction in vivo, in both UV- and MMS-treated conditions (Figures 4D and ^{S2B}). These results suggest that sumoylation unlikely influences Saw1 protein stability or its known interactions with Rad1 and Y-form DNA.

Saw1 Contributes to UV Resistance Independently of NER and Homologous Recombination

The observation that sumoylation of Saw1 does not affect the above properties raised the possibility that its effect could be through mechanisms not hitherto associated with Saw1. We first assessed whether Saw1's effect in the UV situation is related to two main UV lesion removal pathways, Rad51-mediated homologous recombination (HR) and NER (Krogh and Symington, 2004; Schäfer, 2013). In each case, we examined the combinatorial mutant between *saw1-K221R* or *saw1* with the null of representative proteins of the pathway, *saw1-K221R* and *saw1* sensitized mutants that either lack Rad55 and Rad57 in the Rad51-mediated recombination pathway (Figures 5A, 5C, and ^{S3}) or lack Rad26 and Rad7-Rad16 in the two branches of NER (Figures 5B, 5D, and ^{S3}). These results suggest that UV resistance mediated by Saw1 and its sumoylation is separable from Rad51-dependent HR or NER.

SUMO Favors Saw1 Interaction with Slx1-Slx4, and the Two Are Epistatic in the UV Situation

Because Saw1 is a scaffold for the Rad1-Rad10 nuclease, we queried whether Saw1 interacts with other structure-specific nucleases. An interaction with Slx4 was detected in both yeast two-hybrid and in vitro pull-down assays (Figures 6A-6B). Slx4 binds to Slx1 to form a nuclease that cleaves 5' flaps with opposite polarity as Rad1-Rad10 (Fricke and Brill, 2003). Although no Saw1-Slx1 interaction was detected in 2H assay, Slx1 showed interaction with SUMO (Figure 6A). The Slx1-SUMO and Slx4-Saw1 interactions suggest a dual interaction mode between SUMO-Saw1 and Slx1-Slx4. In support of this idea, fusing SUMO to Saw1 enhanced Slx4 interaction in two-hybrid assay, compared with Saw1 (Figure 6C). Interestingly, this fusion reduced interaction with Rad1 (Figure 6C). These results suggest competition between Slx4 and Rad1 for Saw1 binding, and that SUMO

favors the former at the expense of the latter. Consistent with this notion, the Saw1- RBD mutant that cannot interact with Rad1 showed stronger interaction with Slx4 than its wild-type counterpart (Figures 6 and S4). Taken together, our results suggest that SUMO could act as a switch to favor Saw1 interaction with Slx4 over Rad1.

We next examined whether the SUMO-enhanced Saw1-Slx4 interaction pertains to the UV situation using epistasis analysis. Figure 6D shows that *slx4* cells reproducibly showed slightly more sensitivity than wild-type cells in the higher UV dose range, and that the *saw1 slx4* double mutant behaved like the *saw1* single mutant. This genetic relationship supports a functional relationship between Saw1 and Slx4 in the UV condition.

DISCUSSION

Saw1 is a recently identified DNA repair scaffold protein that recruits the Rad1 -Rad10 nuclease to flap DNA during SSA repair of DNA breaks (Li et al., 2008,2013). Here, we show that Saw1 also contributes to survival in the presence of other types of DNA lesions. Its roles in situations that require the repair of base lesions and Top1 -DNA adducts depend on Rad1 and flap binding, as in the case of SSA. We thus propose that Saw1 recruits Rad1-Rad10 to flap DNA in multiple repair contexts, both as those tested here and possibly others that require Rad1-Rad 10 flap cleavage, such as recombination between dispersed repeats or synthesis-dependent strand annealing (Diamante et al., 2014; Mazón et al., 2012) (Figure 6E).

Distinct from these processes, Saw1's role in the UV situation only partially depends on Rad1 binding, and not on flap binding. These results suggest that Saw1 uses a distinct mechanism in this situation, likely involving interaction with different DNA structures and nucleases. As Saw1 binds to DNA bubbles (Li et al., 2013), this interaction may contribute to UV repair when the region of local distortion caused by bulky photoproducts is unwound. One candidate nuclease that Saw1 collaborates with is Slx1-Slx4. The observed Saw1-Slx4 and Slx1-SUMO interactions suggest a two-pronged interaction mode to confer binding specificity to the sumoylated form of Saw1 for the nuclease. In addition, we found that SUMO favors the Saw1-Slx4 interaction at the expense of the Saw1-Rad1 interaction. These results suggest a SUMO-based switch of Saw1 binding partner toward Slx4. As *saw1* is epistatic with *slx4* for UV sensitivity, Saw1 can partly collaborate with Slx1-Slx4 in UV repair. However, as *slx4* is not as sensitive to UV as *saw1*, Saw1 may have other nuclease partners or other roles. Though these roles are currently unclear, our data suggest that they are genetically separable from SSA, Rad51-dependent HR, and NER. Although lesion tolerance mechanisms are candidates, an interesting possibility is that Saw1 may be part of an alternative excision repair pathway, which mimics a minimal UV excision repair pathway found in fission yeast and *N. crassa* (Bowman et al., 1994; McCready et al., 2000; Takao et al., 1996; Yajima et al., 1995; Yasui, 2013; Yonemasu et al., 1997). In this scenario, Saw1 and its sumoylation may coordinate Slx1-Slx4 and Rad1-Rad10 for the cleavage reaction in this repair (Figure 6E).

It is noteworthy that mammalian SLX4 contains a large N-terminal extension that is absent in the yeast Slx4 protein. This extended region interacts with the Rad1-Rad10 homolog

(ERCC4-ERCC1), whereas the conserved region interacts with SLX1 (Figure S5) (Fekairi et al., 2009; Muñoz et al., 2009; Svendsen et al., 2009). Dual nuclease interaction in this case may be functionally similar to the Saw1 interactions with Rad1-Rad10 and Slx1-Slx4 in yeast. This raises the possibility that Saw1 serves the function of the N-terminal region of mammalian SLX4. In both cases, the scaffolds assist their associated nucleases in multiple molecular settings. Further testing of this notion will shed light on the evolutionarily important mechanisms in scaffold-mediated nuclease coordination.

Our findings expand the roles of SUMO in coping with UV lesions beyond the previously reported effects on Rad1 and XRCC1 (Sarangi et al., 2014; Wang et al., 2005). Unique to this case, sumoylation dictates a specific function for Saw1, rather than affecting general protein attributes. This is an example of SUMO specifying a DNA repair factor to a particular function. Our findings suggest one possible mechanism involving Slx1-Slx4 and rule out several others. As our understanding of Saw1 function in UV repair grows, this and additional mechanisms can be tested thoroughly. In conclusion, our findings highlight the versatility of Saw1 as a nuclease scaffold in promoting cell survival in different genotoxic stress conditions and reveal an additional role for sumoylation in promoting UV resistance. These findings open up avenues to explore the roles of this nuclease scaffold in DNA repair.

EXPERIMENTAL PROCEDURES

Yeast Strains and Genetic Manipulations

Strains used are listed in Table 1. Standard yeast protocols were used for strain generation, growth, medium preparation, and DNA damage sensitivity assays. For DNA damage sensitivity tests, log phase cells were diluted 10- or 3-fold and spotted onto YPD media with or without MMS or CPT, or irradiated with UV. For UV treatment, cells were irradiated on plates, and all subsequent steps were done in conditions that prevent light exposure. For survival curves, colonies were counted after incubation for 48 hr. For spot assays, plates were incubated at 30°C and photographed after 24–72 hr. Yeast two hybrid assays were performed as described (Hang et al., 2011). Note that 3AT was added to SC–L-T-H media to detect only the stronger two hybrid interactions (Joung et al., 2000).

Detection of Sumoylated Proteins and Immunoprecipitation

These were performed as described previously (Cremona et al., 2012). In brief, cells were lysed by bead beating in denaturing conditions and TAP-tagged proteins were immunoprecipitated using immunoglobulin (Ig) G-Sepharose. These were washed and eluted with loading dye, followed by SDS-PAGE and western blotting with antibodies against SUMO and the protein A part of the TAP tag (Sigma-Aldrich). Damage-induced sumoylation was assessed by exposing log-phase cells to 100 or 200 J/m² UV using UV Stratalinker 1800 (Stratagene), 0.3% methylmethane sulfonate (MMS, Sigma-Aldrich), or 50 µg/ml camptothecin (CPT, Sigma-Aldrich) for 2 hr. We note that, unlike most sumoylated proteins characterized thus far whose sumoylation levels are very low (Ulrich, 2009), sumoylation of Saw1 can be readily detected by the antibody against the tag (Figure 2C). Quantification of the bands showed that approximately 7% of Saw1 is sumoylated under normal growth conditions and around 26% after damage treatment. This makes Saw1

one of the rare substrates with high levels of sumoylation. Coimmunoprecipitation was done as described previously (Hang et al., 2011).

His₆- and GST-Saw1 Protein Purification

The plasmid expressing Saw1 protein with (His)₆-affinity tag was introduced into *E. coli* strain Rosetta (DE3) pLysS. Protein expression was induced by 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) at 37°C for 4 hr. Extract from 13 g of cell paste was prepared by sonication in 50 ml of buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10% glycerol, 0.5% Triton X-100, 1 mM β-mercaptoethanol, and protease inhibitor cocktail. The lysate was clarified by ultracentrifugation, and the resulting supernatant was incubated with 1 ml Ni-NTA agarose (QIAGEN) for 2 hr at 4°C. The beads were washed with 12 ml of buffer T (25 mM Tris-HCl, 10% glycerol, 0.5 mM EDTA [pH 7.5]) containing 100 mM KCl. The bound proteins were eluted with buffer T containing 50 mM KCl and imidazole (from 50 to 1,000 mM). Fractions containing Saw1 (500–1,000 mM imidazole) were applied onto a 0.5 ml MonoS column (GE Healthcare), and eluted using 200–1,000 mM KCl in buffer T. The peak Saw1 fractions (550–1,000 mM KCl) were concentrated to 3 μg/μl in a Vivaspin-2 concentrator. For GST-Saw1, the *E. coli* strain BL21(DE3)pLysS was transformed with a plasmid expressing GST-tagged Saw1 protein. Protein expression was induced by addition of 0.1 mM IPTG at 16°C overnight. Ten grams of cell paste was sonicated in 50 ml of buffer CBB (50 mM Tris-HCl [pH 7.5], 10% sucrose, 2 mM EDTA, 150 mM KCl, 0.01% NP40, 1 mM DTT, and protease inhibitor cocktail). The lysate was clarified by ultracentrifugation and the supernatant was loaded on a 7-ml Sp-Sepharose column (GE Healthcare). The column was eluted using 150–1,000 mM KCl in buffer K (20 mM K₂HPO₄, 10% glycerol, 0.5 mM EDTA [pH 7.5]). Peak Saw1 fractions eluting around 400–600 mM KCl were incubated with 700 μl glutathione-Sepharose (GE Healthcare) for 1 hr at 4°C. The beads were washed with 10 ml of buffer K containing 100 mM KCl and eluted in steps with 50–200 mM glutathione in buffer K containing 100 mM KCl. The fractions containing Saw1 (100–200 mM glutathione) were applied onto a 1 ml MonoS column (GE Healthcare) and eluted using 200–1,000 mM KCl in buffer K. The peak fractions (500–800 mM KCl) were concentrated to 10 μg/μl in a Vivaspin-2 concentrator. The *saw1-K221R* mutant was generated by site-directed mutagenesis.

Pull-Down Assay

Purified GST-Saw1 (3 μM) and Slx4 (0.2 μM) proteins were incubated with 25 μl of glutathione-Sepharose 4 Fast Flow (GE Healthcare) in 25 μl of buffer T (20 mM Tris-HCl [pH 7.5], 80 mM KCl, 1 mM DTT, 0.5 mM EDTA, and 0.01 % NP40) for 30 min at 4°C, with gentle shaking. Following incubation, the supernatants were collected and mixed with 20 μl of SDS Laemmli buffer. After washing the beads with 100 μl of buffer T, the bound proteins were eluted with 30 μl of SDS Laemmli buffer. The supernatant and SDS eluate fractions were subjected to SDS-PAGE analysis.

Other Assays

In vitro sumoylation assay, mass spectrometry detection of sumoylated lysines, and electrophoretic mobility shift assays (EMSA) were performed as described previously,

except that the EMSA used a 5% polyacrylamide gel in $0.5 \times$ Tris-borate-EDTA and 6 nM DNA substrate (Sarangi et al., 2014). His-tagged Slx4 was purified as described (Fricke and Brill, 2003). Chromosomal SSA assay was performed as described earlier (Li et al., 2008). In brief, log phase cells were grown in YP-glycerol and then plated on YP-glucose or YP-galactose plates, and colonies were counted after 3–4 days. Percentage survival was calculated as number of colonies on YP-galactose plates divided by that on YP-glucose plates.

ACKNOWLEDGMENTS

We thank Steve Brill for the Slx4 expression plasmid. We are grateful to X.Z. lab members for discussions and useful suggestions. This work is supported by NIH grant GM071011 to S.E.L.; Czech Science Foundation (GACR 13-26629S and 207/12/2323), European Regional Development Fund (Project FNUSA-ICRC; no. CZ.1.05/1.1.00/02.0123) and CZ.1.07/2.3.00/20.0011 cofinanced by European Social Fund and the state budget of the Czech Republic to L.K.; “Employment of Newly Graduated Doctors of Science for Scientific Excellence” (CZ.1.07/2.3.00/30.0009) cofinanced by European Social Fund to V.A.; NIH grant GM080670, American Cancer Society grant RSG-12-013-01-CCG, and Leukemia and Lymphoma Society Scholar Award to X.Z. Funding for open access charge was provided by the NIH.

REFERENCES

- Altmannova V, Eckert-Boulet N, Arneric M, Kolesar P, Chaloupkova R, Damborsky J, Sung P, Zhao X, Lisby M, Krejci L. Rad52 SUMOylation affects the efficiency of the DNA repair. *Nucleic Acids Res.* 2010; 38:4708–4721. [PubMed: 20371517]
- Bai Y, Symington LS. A Rad52 homolog is required for RAD51-independent mitotic recombination in *Saccharomyces cerevisiae*. *Genes Dev.* 1996; 10:2025–2037. [PubMed: 8769646]
- Boiteux S, Guillet M. Abasic sites in DNA: repair and biological consequences in *Saccharomyces cerevisiae*. *DNA Repair (Amst.)*. 2004; 3:1–12. [PubMed: 14697754]
- Bowman KK, Sidik K, Smith CA, Taylor JS, Doetsch PW, Freyer GA. A new ATP-independent DNA endonuclease from *Schizosaccharomyces pombe* that recognizes cyclobutane pyrimidine dimers and 6–4 photoproducts. *Nucleic Acids Res.* 1994; 22:3026–3032. [PubMed: 8065916]
- Chen YH, Szakal B, Castellucci F, Branzei D, Zhao X. DNA damage checkpoint and recombinational repair differentially affect the replication stress tolerance of Smc6 mutants. *Mol. Biol. Cell.* 2013; 24:2431–2441. [PubMed: 23783034]
- Cremona CA, Sarangi P, Yang Y, Hang LE, Rahman S, Zhao X. Extensive DNA damage-induced sumoylation contributes to replication and repair and acts in addition to the mec1 checkpoint. *Mol. Cell.* 2012; 45:422–432. [PubMed: 22285753]
- Diamante G, Phan C, Celis AS, Krueger J, Kelson EP, Fischhaber PL. *SAWI* is required for SDSA double-strand break repair in *S. cerevisiae*. *Biochem. Biophys. Res. Commun.* 2014; 445:602–607. [PubMed: 24565838]
- Fekairi S, Scaglione S, Chahwan C, Taylor ER, Tissier A, Coulon S, Dong MQ, Ruse C, Yates JR 3rd, Russell P, et al. Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/ recombination endonucleases. *Cell.* 2009; 138:78–89. [PubMed: 19596236]
- Fishman-Lobell J, Rudin N, Haber JE. Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. *Mol. Cell. Biol.* 1992; 12:1292–1303. [PubMed: 1545810]
- Fricke WM, Brill SJ. Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. *Genes Dev.* 2003; 17:1768–1778. [PubMed: 12832395]
- Guillet M, Boiteux S. Endogenous DNA abasic sites cause cell death in the absence of Apn1, Apn2 and Rad1/Rad10 in *Saccharomyces cerevisiae*. *EMBO J.* 2002; 21:2833–2841. [PubMed: 12032096]
- Guzder SN, Sommers CH, Prakash L, Prakash S. Complex formation with damage recognition protein Rad14 is essential for *Saccharomyces cerevisiae* Rad1-Rad10 nuclease to perform its function in nucleotide excision repair *in vivo*. *Mol. Cell. Biol.* 2006; 26:1135–1141. [PubMed: 16428464]

- Hammel M, Rey M, Yu Y, Mani RS, Classen S, Liu M, Pique ME, Fang S, Mahaney BL, Weinfeld M, et al. XRCC4 protein interactions with XRCC4-like factor (XLF) create an extended grooved scaffold for DNA ligation and double strand break repair. *J. Biol. Chem.* 2011; 286:32638–32650. [PubMed: 21775435]
- Hang LE, Liu X, Cheung I, Yang Y, Zhao X. SUMOylation regulates telomere length homeostasis by targeting Cdc13. *Nat. Struct. Mol. Biol.* 2011; 18:920–926. [PubMed: 21743457]
- Heyer WD, Ehmsen KT, Liu J. Regulation of homologous recombination in eukaryotes. *Annu. Rev. Genet.* 2010; 44:113–139. [PubMed: 20690856]
- Johnson ES, Gupta AA. An E3-like factor that promotes SUMO conjugation to the yeast septins. *Cell.* 2001; 106:735–744. [PubMed: 11572779]
- Joung JK, Ramm EI, Pabo CO. A bacterial two-hybrid selection system for studying protein-DNA and protein-protein interactions. *Proc. Natl. Acad. Sci. USA.* 2000; 97:7382–7387. [PubMed: 10852947]
- Krogh BO, Symington LS. Recombination proteins in yeast. *Annu. Rev. Genet.* 2004; 38:233–271. [PubMed: 15568977]
- Li F, Dong J, Pan X, Oum JH, Boeke JD, Lee SE. Micro-array-based genetic screen defines *SAW1*, a gene required for Rad1/Rad10-dependent processing of recombination intermediates. *Mol. Cell.* 2008; 30:325–335. [PubMed: 18471978]
- Li F, Dong J, Eichmiller R, Holland C, Minca E, Prakash R, Sung P, Yong Shim E, Surtees JA, Eun Lee S. Role of Saw1 in Rad1/Rad10 complex assembly at recombination intermediates in budding yeast. *EMBO J.* 2013; 32:461–472. [PubMed: 23299942]
- Mazón G, Lam AF, Ho OK, Kupiec M, Symington LS. The Rad1-Rad10 nuclease promotes chromosome translocations between dispersed repeats. *Nat. Struct. Mol. Biol.* 2012; 19:964–971. [PubMed: 22885325]
- McCready SJ, Osman F, Yasui A. Repair of UV damage in the fission yeast *Schizosaccharomyces pombe*. *Mutat. Res.* 2000; 451:197–210. [PubMed: 10915873]
- Muñoz IM, Hain K, Déclais AC, Gardiner M, Toh GW, Sanchez-Pulido L, Heuckmann JM, Toth R, Macartney T, Eppink B, et al. Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. *Mol. Cell.* 2009; 35:116–127. [PubMed: 19595721]
- Pouliot JJ, Yao KC, Robertson CA, Nash HA. Yeast gene for a Tyr-DNA phosphodiesterase that repairs topoisomerase I complexes. *Science.* 1999; 286:552–555. [PubMed: 10521354]
- Prolla TA, Pang Q, Alani E, Kolodner RD, Liskay RM. MLH1, PMS1, and MSH2 interactions during the initiation of DNA mismatch repair in yeast. *Science.* 1994; 265:1091–1093. [PubMed: 8066446]
- Psakhye I, Jentsch S. Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. *Cell.* 2012; 151:807–820. [PubMed: 23122649]
- Sarangi P, Bartosova Z, Altmannova V, Holland C, Chavdarova M, Lee SE, Krejci L, Zhao X. Sumoylation of the Rad1 nuclease promotes DNA repair and regulates its DNA association. *Nucleic Acids Res.* 2014; 42:6393–6404. [PubMed: 24753409]
- Schärer OD. Nucleotide excision repair in eukaryotes. *Cold Spring Harb. Perspect. Biol.* 2013; 5:a012609. [PubMed: 24086042]
- Svensden JM, Smogorzewska A, Sowa ME, O'Connell BC, Gygi SP, Elledge SJ, Harper JW. Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. *Cell.* 2009; 138:63–77. [PubMed: 19596235]
- Takahashi Y, Toh-e A, Kikuchi Y. A novel factor required for the SUMO1/Smt3 conjugation of yeast septins. *Gene.* 2001; 275:223–231. [PubMed: 11587849]
- Takao M, Yonemasu R, Yamamoto K, Yasui A. Characterization of a UV endonuclease gene from the fission yeast *Schizosaccharomyces pombe* and its bacterial homolog. *Nucleic Acids Res.* 1996; 24:1267–1271. [PubMed: 8614629]
- Ulrich HD. The SUMO system: an overview. *Methods Mol. Biol.* 2009; 497:3–16. [PubMed: 19107407]
- Vance JR, Wilson TE. Yeast Tdp1 and Rad1-Rad10 function as redundant pathways for repairing Top1 replicative damage. *Proc. Natl. Acad. Sci. USA.* 2002; 99:13669–13674. [PubMed: 12368472]

- Vidal AE, Boiteux S, Hickson ID, Radicella JP. XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. *EMBO J.* 2001; 20:6530–6539. [PubMed: 11707423]
- Wang QE, Zhu Q, Wani G, El-Mahdy MA, Li J, Wani AA. DNA repair factor XPC is modified by SUMO-1 and ubiquitin following UV irradiation. *Nucleic Acids Res.* 2005; 33:4023–4034. [PubMed: 16030353]
- Yajima H, Takao M, Yasuhira S, Zhao JH, Ishii C, Inoue H, Yasui A. A eukaryotic gene encoding an endonuclease that specifically repairs DNA damaged by ultraviolet light. *EMBO J.* 1995; 14:2393–2399. [PubMed: 7774597]
- Yasui A. Alternative excision repair pathways. *Cold Spring Harb. Perspect. Biol.* 2013; 5:a012617.
- Yonemasu R, McCready SJ, Murray JM, Osman F, Takao M, Yamamoto K, Lehmann AR, and Yasui A. Characterization of the alternative excision repair pathway of UV-damaged DNA in *Schizosaccharomyces pombe*. *Nucleic Acids Res.* 1997; 25:1553–1558. [PubMed: 9092661]
- Zhao X, Blobel G. A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. *Proc. Natl. Acad. Sci. USA.* 2005; 102:4777–4782. [PubMed: 15738391]

Highlights

The Saw1 scaffold has multiple roles and copes with diverse types of DNA lesions

Saw1 assists the Rad1 -Rad10 nuclease in a range of DNA damage conditions

Sumoylation of Saw1 facilitates its interaction with another nuclease Slx1 -Slx4

Saw1 sumoylation promotes UV resistance independently of two repair pathways

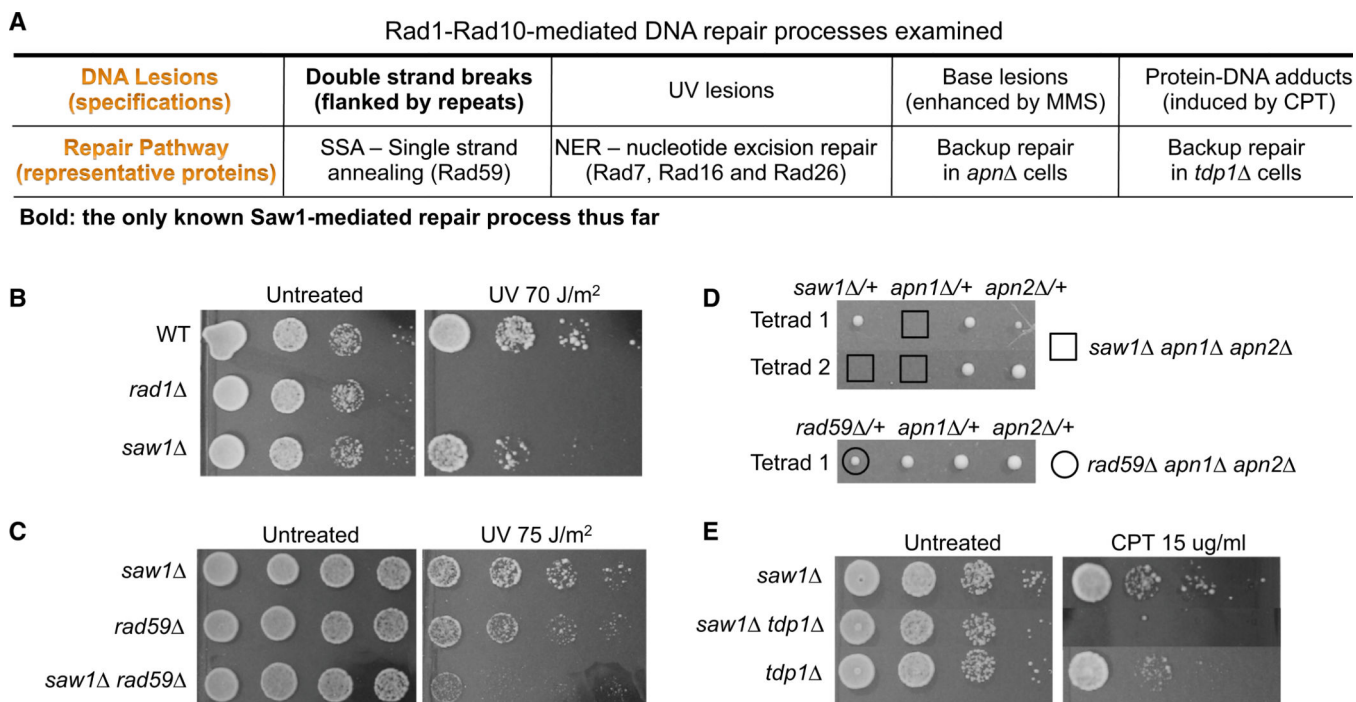


Figure 1. Saw1 Promotes Resistance to Multiple Types of DNA Lesions

(A) Summary of Rad1-Rad10-mediated DNA repair processes relevant to this study.

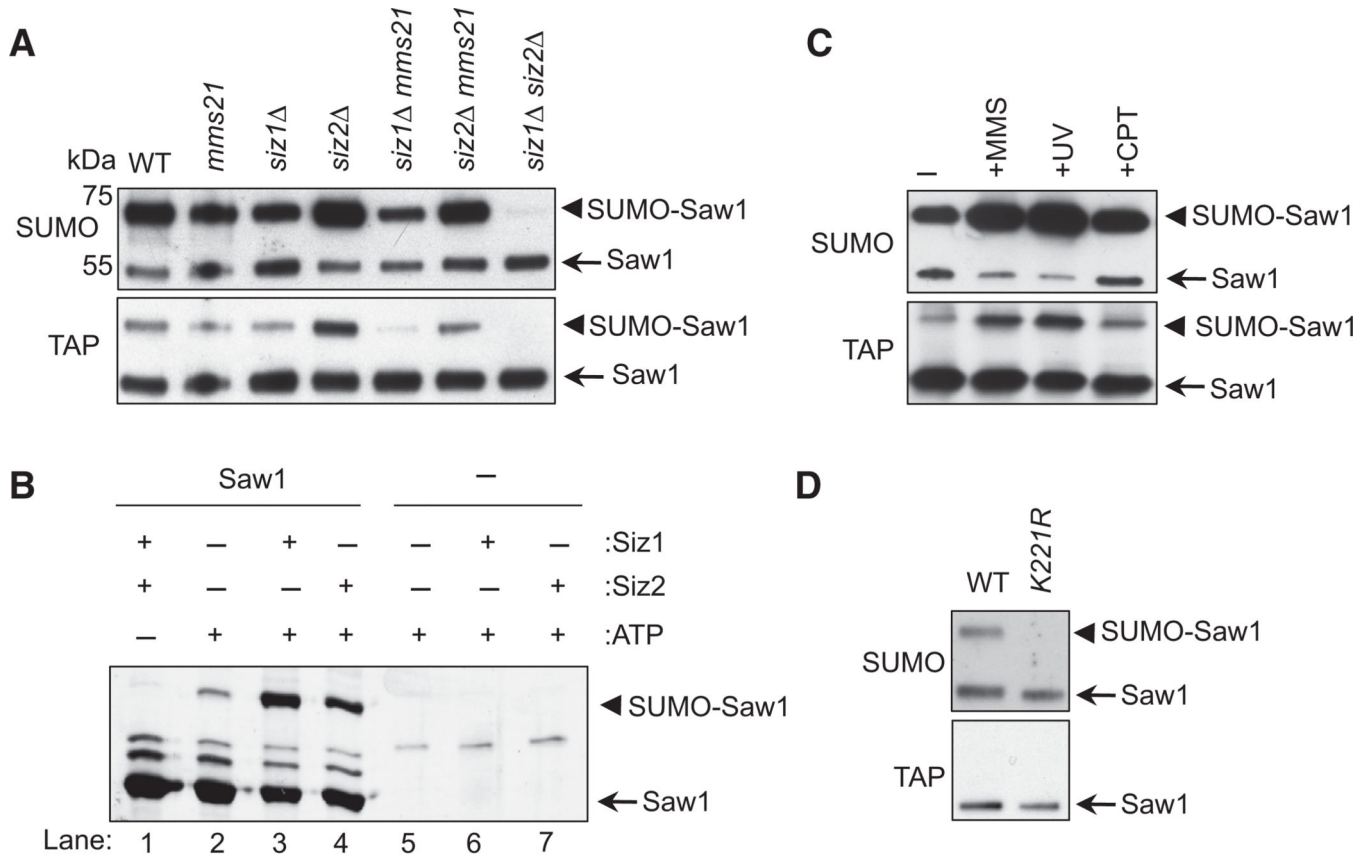
(B) *saw1* and *rad1* cells are sensitive to UV radiation.

(C) *saw1* sensitizes *rad59* to UV.

(D) *apn1 apn2* is synthetically lethal with *saw1* but not *rad59*. Representative tetrads dissected from diploids with indicated genotypes are shown. Triple mutants are labeled and spore clones of other genotypes grow similarly.

(E) *saw1* enhances the CPT sensitivity of *tdp1* cells.

In (B) and (E), 10-fold serial dilutions of cell cultures were spotted and either untreated or treated with the indicated UV dose (B) or on media containing CPT(E). In (C), 3-fold dilutions were used.



All reactions contain SUMO, SUMO E1 and E2

Figure 2. Saw1 Monosumoylation Increases after DNA Damage Treatment

(A) Saw1 sumoylation level in SUMO ligase mutants. Indicated strains were treated with UV and immunoprecipitated Saw1-TAP was examined by western blotting using antibody recognizing TAP (bottom) and SUMO (top). Note that the modified form of Saw1 runs ~20 kDa higher than the unmodified form on SDS-PAGE gels, a signature shift caused by sumoylation.

(B) In vitro sumoylation of Saw1 is stimulated by the SUMO E3s Siz1 and Siz2.

Recombinant Saw1 was subjected to standard in vitro sumoylation reactions; all lanes have SUMO (Smt3), E1 (Aos1/ Uba2), and E2 (Ubc9). Saw1 sumoylation in the absence of E3 and presence of ATP is due to Ubc9-mediated direct conjugation and is further stimulated by the Siz1 and Siz2 E3s.

(C) Saw1 sumoylation is induced by MMS, UV, and CPT treatment. Saw1 sumoylation in cells treated with different DNA damaging agents was examined as in (A). Note that the increased sumoylation of Saw1 after DNA damage treatment can be seen on both blots.

(D) Saw1-K221R is not sumoylated in vivo. Indicated strains were examined for Saw1 sumoylation after exposure to 100 J/m² UV.

See also Figure S1.

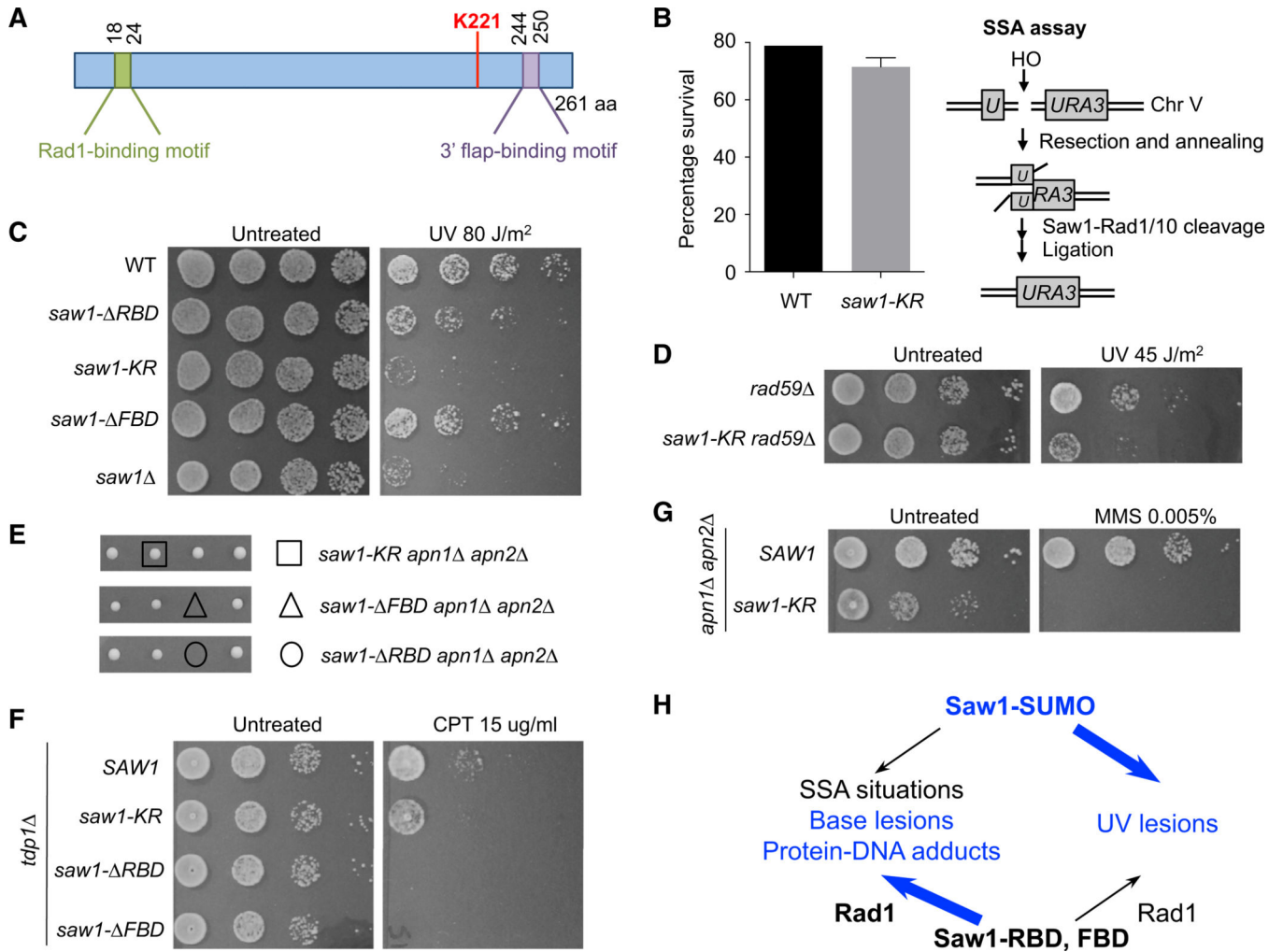


Figure 3. Differential Effects of Saw1 Attributes under Several Damage Situations
 (A) Schematic of Saw1 depicting three main features. Motifs required for binding to Rad1 and flap DNA and sumoylation site are shown.
 (B) *saw1-K221R* is proficient for SSA repair. Schematic of SSA assay is on the right. *saw1-K221R* is denoted as *saw1-KR* here and in other panels. Data from three trials are represented as mean ± SD.
 (C) *saw1-K221R* behaves like *saw1* and is more sensitive to UV than *saw1-RBD* and *saw1-FBD*. As in Figure 1B, 3-fold serial dilutions were spotted.
 (D) *saw1-K221R* is additive with *rad59* for UV sensitivity. As in Figure 1A, 10-fold serial dilutions were spotted.
 (E) *saw1-RBD* and *saw1-FBD*, but not *saw1-K221R*, are synthetically lethal with *apn1 apn2*. Diploids heterozygotic for the indicated mutations were dissected, and a representative tetrad is shown for each diploid. Double mutants are labeled.
 (F) *saw1-RBD* and *saw1-FBD* cells exhibit stronger sensitization of *tdp1* than *saw1-K221R* on CPT. As in Figure 1E, 3-fold serial dilutions were spotted.
 (G) *saw1-K221R* slows *apn1 apn2* cell growth and exacerbates its MMS sensitivity.

(H) Schematic depicting the different contributions of the three Saw1 attributes to its functions under diverse DNA damage conditions. Newly found contributions are in blue. Thicker lines indicate greater contributions.

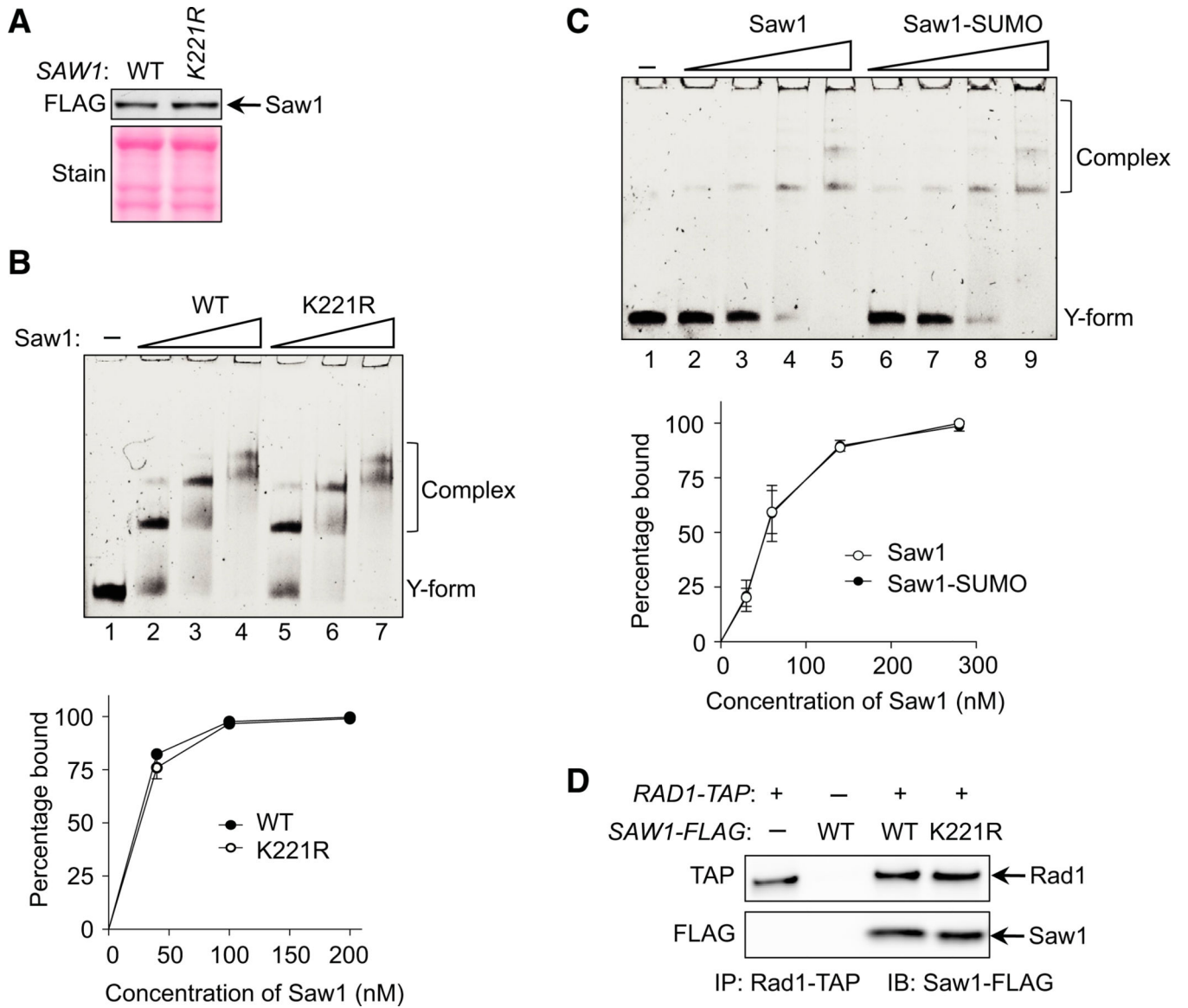


Figure 4. Saw1 Sumoylation Affects Neither Its Protein Level nor Its Interactions with Rad1 and Y-Form DNA

(A) *saw1-K221R* does not affect Saw1 protein level after UV treatment. Extracts from cells with Saw1 and Saw1-K221R tagged with FLAG at its own chromosomal locus were examined by western blotting (top). Loading is shown on the bottom.

(B) Saw1-K221R is indistinguishable from wild-type protein for binding to Y-form DNA. Increasing concentrations of recombinant wild-type (lanes 2–4) and mutant Saw1 (lanes 5–7) (30–280 nM) were tested by EMSA for binding to Y-form DNA (6 nM). Protein-DNA binding is manifested by the upshift of the fluo-rescently labeled DNA (complex). Percentages of Y-form DNA shifted from three trials were quantified as mean ± SD (bottom).

(C) Sumoylation of Saw1 does not alter interaction with Y-form DNA in vitro. Recombinant GST-Saw1 was subjected to in vitro sumoylation as in Figure 2B to yield about 40% sumoylated Saw1. The mixture of the products (40–200 nM) was tested for binding to Y-

form DNA (6 nM) (lanes 6–9) and compared with similar amounts of Saw1 that underwent the same procedure in the absence of SUMO E1 (lanes 2–5). Percentages of Y-form shifted from three trials were quantified as mean \pm SD (bottom). Note that the different DNA shift pattern here compared with that in (B) is likely due to changes caused by incubation for sumoylation reactions or other proteins in the reactions.

(D) Saw1 -K221R is proficient for Rad1 interaction in vivo after UV treatment. Rad1 -TAP was pulled down, and coimmunoprecipitated Saw1 -FLAG was detected by antibody against FLAG. The ratio of copurified Saw1 to Rad1 is similar between wild-type and *saw1-K221R* cells.

See also Figure S2.

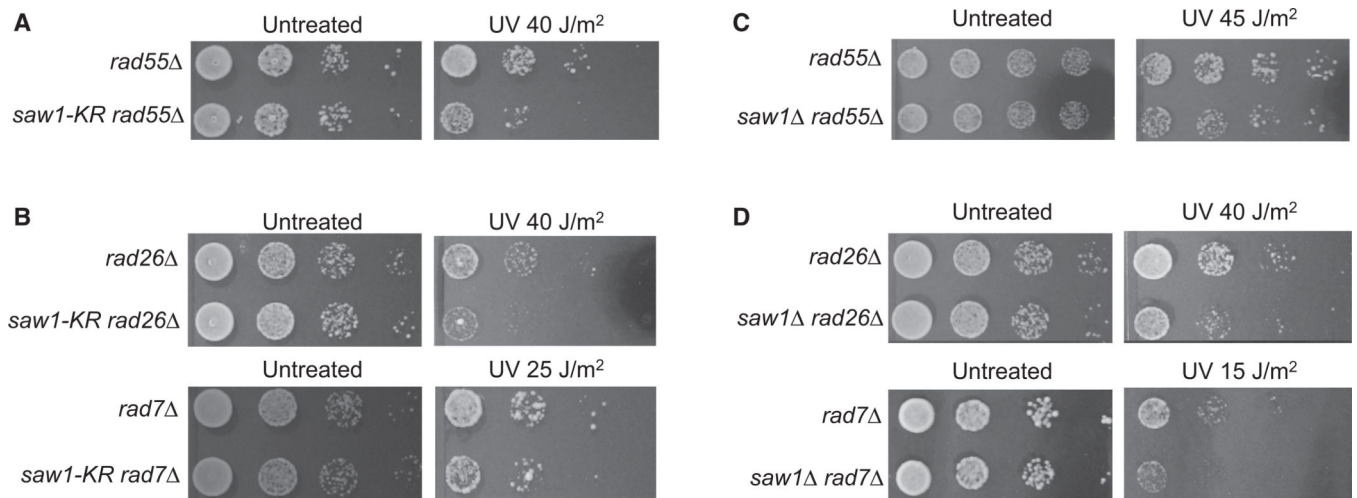


Figure 5. Saw1 and Its Sumoylation Contribute to UV Resistance Independently of Rad51-Dependent HR and NER

(A–D) *saw1* and *saw1-K221R* exacerbate the UV sensitivities of *rad55*, *rad26*, and *rad7A* cells. In (A) and (B), cells were spotted in 10-fold serial dilutions; in (C) and (D), 3-fold serial dilutions were used. Note that *saw1* mutant alone does not exhibit noticeable sensitivity at the UV doses shown. See also Figure S3.

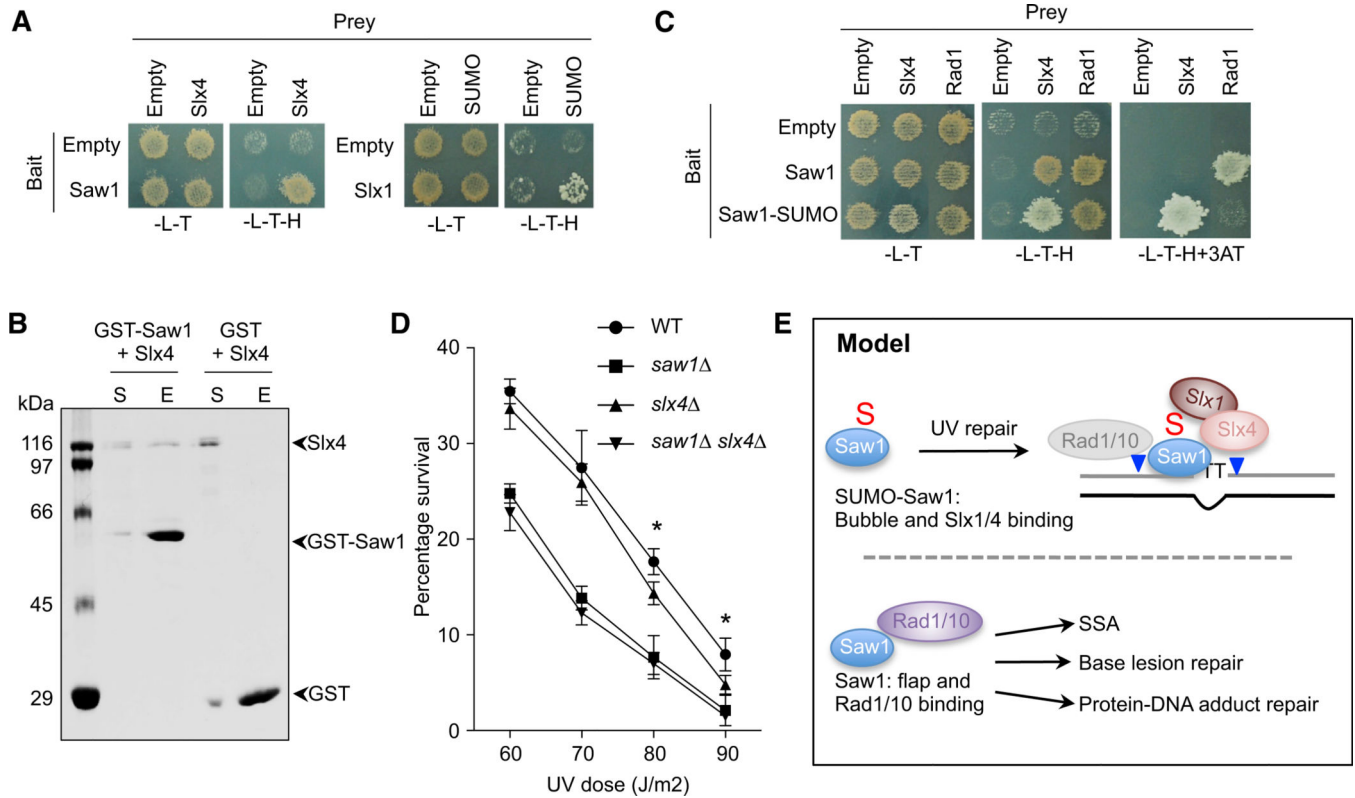


Figure 6. Saw1 Physical and Genetic Interactions with Slx4

(A) Saw1 interacts with Slx4 and SUMO interacts with Slx1 in yeast two-hybrid assay. Cells transformed with the indicated plasmids were patched onto selection plates. Growth on SC-L-T plates indicates presence of plasmids, and growth on SC-L-T-H plates indicates interaction.

(B) GST-Saw1, but not GST, pulls down Slx4 in vitro. Supernatant (S) and eluate (E) of each of the GST pull-down reactions are shown.

(C) Fusing SUMO to the C terminus of Saw1 enhances Slx4 interaction and reduces Rad1 interaction. Similar to (A), growth on SC-L-T-H+3AT indicates stronger interaction.

(D) *saw1Δ* is epistatic to *slx4Δ* for UV sensitivity. Data from at least three trials are represented as mean \pm SD. Asterisks denote statistically significant differences between survival of wild-type and *slx4Δ* cells ($p < 0.05$).

(E) Top: possible model for Saw1 and its sumoylation in promoting UV repair. This could involve Saw1 binding to bubble DNA structures and SUMO-enhanced binding of Saw1 to the Slx1-Slx4 nuclease. Saw1 interaction with Rad1-Rad10 plays minor roles here, and the two nucleases may be coordinated for dual cleavage of the DNA lesion. Bottom: Saw1 and its ability to bind Rad1 and 3'flap DNA are required for SSA and likely the repair of base lesions and protein-DNA adducts. Note that for simplicity, partial contributions of Saw1 sumoylation to the latter two repair processes are not drawn.

See also Figure S4.

Table 1

Yeast Strains Used in This Study

Strain	Genotype
W1588-4A	<i>MATalpha ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 RAD5</i>
X3401-1C	<i>MATalpha rad1 ::LEU2</i>
T956-1	<i>MATalpha saw1 ::KAN</i>
X5318-9B	<i>rad59 ::LEU2</i>
X5318-11B	<i>saw1 ::KAN rad59 ::LEU2</i>
X5316-1A	<i>saw1 ::KAN tdp1 ::KAN</i>
T958-3	<i>SAW1-TAP::HIS3</i>
X4505-3A	<i>SAW1-TAP::HIS3 siz1 ::KAN</i>
X4505-5A	<i>SAW1-TAP::HIS3 siz2 ::URA3</i>
X4506-9A	<i>SAW1-TAP::HIS3 mms21-11::HIS3</i>
X4506-9D	<i>SAW1-TAP::HIS3 siz1 ::KAN mms21-11::HIS3</i>
X4507-1A	<i>SAW1-TAP::HIS3 siz2 ::URA3 mms21-11::HIS3</i>
X4505-2D	<i>SAW1-TAP::HIS3 siz1 ::KAN siz2 ::URA3</i>
T1490-2	<i>saw1-K221R-TAP::HIS3</i>
X5314-1A	<i>saw1- RBD-TAP::HIS3</i>
X5313-1A	<i>saw1- FBD-TAP::HIS3</i>
X5519-1C	<i>SAW1-TAP::HIS3 rad59 ::LEU2</i>
X5624-1A	<i>saw1-K221R-TAP::HIS3 rad59 ::LEU2</i>
SLY5151	<i>ho HML mat::leu2::hisG hmr 3 leu2-3,112 ura3-52 trp1 THR4-ura3-A(205bp)-HOcs-URA3-A ade3::GAL10-HO::NAT</i>
X5638-11B	<i>SAW1-TAP::HIS3 apn1 ::KAN apn2 ::HIS3</i>
X5639-7C	<i>saw1-K221R-TAP::HIS3 apn1 ::KAN apn2 ::HIS3</i>
X5359-9A	<i>SAW1-TAP::HIS3 tdp1 ::KAN</i>
X5360-5A	<i>saw1-K221R-TAP::HIS3 tdp1 ::KAN</i>
X5643-2C	<i>saw1- RBD-TAP::HIS3 tdp1 ::KAN</i>
X5644-8A	<i>saw1- FBD-TAP::HIS3 tdp1 ::KAN</i>
X4965-2D	<i>RAD1-TAP::HIS3</i>
X4965-2B	<i>SAW1-3FLAG::KAN</i>
X4965-2C	<i>RAD1-TAP::HIS3 SAW1-3FLAG::KAN</i>
X4967-6B	<i>RAD1-TAP::HIS3saw1-K221R-3FLAG::KAN</i>
X5536-6A	<i>rad55 ::KAN</i>
X5535-5A	<i>saw1 ::KAN rad55 ::KAN</i>
X5530-2D	<i>rad26 ::KAN</i>
X5529-1B	<i>saw1 ::KAN rad26 ::KAN</i>
X5532-11A	<i>rad16 ::KAN</i>
X5531-10C	<i>saw1 ::KAN rad16 ::KAN</i>
X5559-3B	<i>SAW1-TAP::HIS3 rad55 ::KAN</i>
X5536-8D	<i>saw1-K221R-TAP::HIS3 rad55 ::KAN</i>
X5561-1D	<i>SAW1-TAP::HIS3 rad26 ::KAN</i>
X5530-2A	<i>saw1-K221R-TAP::HIS3 rad26 ::KAN</i>

Strain	Genotype
X5557-1C	<i>SAW1-TAP::HIS3 rad16 ::KAN</i>
X5532-10B	<i>saw1-K221R-TAP::HIS3 rad16 ::KAN</i>
X5900-3C	<i>slx1 ::KAN</i>
X5899-1B	<i>saw1 ::KAN slx1 ::KAN</i>
X5881-3A	<i>slx4 ::KAN</i>
X5881-3C	<i>saw1 ::KAN slx4 ::KAN</i>

All strains, except those for assaying SSA, are in the W303 background that has wild-type *RAD5*, and the full genotype is listed only for W1588-4A (Chen et al., 2013). Experiments were performed with at least two different spore clones; only one is listed in the table.