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

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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

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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 saw1Δ 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 rad1Δ 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 saw1Δ 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 saw1Δ rad59Δ cells were more UV sensitive than rad59Δ 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 saw1Δ 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 saw1Δ also showed the same genetic interaction with apn1Δ apn2Δ as does rad1Δ. This finding is consistent with the idea that Saw1 is required for the backup repair of base lesions (Figure 1D). This function of Saw1 is separable from SSA, because rad59Δ 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 tdp1Δ rad1Δ cells are inviable on CPT-containing media (Vance and Wilson, 2002). We found that tdp1Δ saw1Δ 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 rad59Δ does not sensitize tdp1Δ 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 Fc 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 siz1Δ siz2A and siz1Δ mms21 double mutants, but not in siz2Δ 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-ΔRBD) or the flap binding (saw1-ΔFBD) 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-ΔRBD, and -ΔFBD 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 saw1Δ. 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 saw1Δ, saw1-K221R sensitized rad59Δ to UV (Figure 3D), further supporting the notion that saw1-K221FTs UV sensitivity is not due to an SSA defect.

Different from saw1-K221R, saw1-ΔFBD showed only slight UV sensitivity, suggesting that flap binding is largely dispensable for UV resistance (Figure 3C). The UV sensitivity of saw1-ΔRBD was in between that of saw1-ΔFBD 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 saw1Δ, saw1-ΔRBD, and -ΔFBD were synthetically lethal with apn1Δ apn2Δ, and strongly sensitized tdp1Δ 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-ΔRBD and -ΔFBD, saw1-K221R apn1Δ apn2Δ cells were viable but exhibited slower growth and stronger MMS sensitivity than apn1Δ apn2Δ (Figures 3E and 3G), and only moderate sensitization of tdp1Δ 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; Schärer, 2013). In each case, we examined the combinatorial mutant between saw1-K221R or saw1Δ with the null of representative proteins of the pathway, saw1-K221R and saw1Δ 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-ΔRBD 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 slx4Δ cells reproducibly showed slightly more sensitivity than wild-type cells in the higher UV dose range, and that the saw1Δ slx4Δ double mutant behaved like the saw1Δ 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-Rad10 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 saw1Δ is epistatic with slx4Δ for UV sensitivity, Saw1 can partly collaborate with Slx1-Slx4 in UV repair. However, as slx4Δ is not as sensitive to UV as saw1Δ, 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).

**His**<sub>6</sub>- and GST-Saw1 Protein Purification**

The plasmid expressing Saw1 protein with (His)<sub>6</sub>-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 K<sub>2</sub>HPO<sub>4</sub>, 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 (EMSAs) were performed as described previously.
except that the EMSA used a 5% polyacrylamide gel in 0.5 × 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.

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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.

| Repair Pathway (representative proteins) | DNA Lesions (specifications) | Double strand breaks (flanked by repeats) | UV lesions | Base lesions (enhanced by MMS) | Protein-DNA adducts (induced by CPT) |
|------------------------------------------|------------------------------|------------------------------------------|------------|---------------------------------|-------------------------------------|
| **Saw1Δ**                                | SSA – Single strand annealing (Rad59) | **NER** – nucleotide excision repair (Rad7, Rad16 and Rad26) | **Backup repair in apn1Δ cells** | **Backup repair in tdp1Δ cells** |

**Bold: the only known Saw1-mediated repair process thus far**

(B) saw1Δ and rad1Δ cells are sensitive to UV radiation.

(C) saw1Δ sensitizes rad59Δ to UV.

(D) apn1Δ apn2Δ is synthetically lethal with saw1Δ but not rad59Δ. Representative tetrads dissected from diploids with indicated genotypes are shown. Triple mutants are labeled and spore clones of other genotypes grow similarly.

(E) saw1Δ enhances the CPT sensitivity of tdp1Δ 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.
**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 saw1Δ and is more sensitive to UV than saw1-ΔRBD and saw1-ΔFBD. As in Figure 1B, 3-fold serial dilutions were spotted.

(D) saw1-K221R is additive with rad59Δ for UV sensitivity. As in Figure 1A, 10-fold serial dilutions were spotted.

(E) saw1-ΔRBD and saw1-ΔFBD, but not saw1-K221R, are synthetically lethal with apn1Δ apn2Δ. Diploids heterozygotic for the indicated mutations were dissected, and a representative tetrad is shown for each diploid. Double mutants are labeled.

(F) saw1-ΔRBD and saw1-ΔFBD cells exhibit stronger sensitization of tdp1Δ than saw1-K221R on CPT. As in Figure 1E, 3-fold serial dilutions were spotted.

(G) saw1-K221R slows apn1Δ apn2Δ 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 ± 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) saw1Δ and saw1-K221R exacerbate the UV sensitivities of rad55Δ, rad26Δ, 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 saw1Δ 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 ± 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.
| Strain   | Genotype                                                                 |
|----------|---------------------------------------------------------------------------|
| W1588-4A | MATalpha ade2-1 his3-11,15 leu2-3,112 trpl-1 ura3-1 can1-100 RAD5          |
| X3401-1C | MATalpha rad1Δ::LEU2                                                      |
| T956-1   | MATalpha saw1Δ::KAN                                                      |
| X3518-9B | rad59Δ::LEU2                                                              |
| X3518-11B| saw1Δ::KAN rad59Δ::LEU2                                                  |
| X3516-1A | saw1Δ::KAN tdp1Δ::KAN                                                     |
| T958-3   | SAW1-TAP::HIS3                                                           |
| X4505-3A | SAW1-TAP::HIS3 siz1Δ::KAN                                                |
| X4505-5A | SAW1-TAP::HIS3 siz2Δ::URA3                                                |
| X4506-9A | SAW1-TAP::HIS3 mms21-11::HIS3                                             |
| X4506-9D | SAW1-TAP::HIS3 siz1Δ::KAN mms21-11::HIS3                                 |
| X4507-1A | SAW1-TAP::HIS3 siz2Δ::URA3 mms21-11::HIS3                                 |
| X4505-2D | SAW1-TAP::HIS3 siz1Δ::KAN siz2Δ::URA3                                    |
| T1490-2  | saw1-K221R-TAP::HIS3                                                    |
| X5314-1A | saw1-ΔRBD-TAP::HIS3                                                      |
| X5313-1A | saw1-ΔFBD-TAP::HIS3                                                      |
| X5519-1C | SAW1-TAP::HIS3 rad59Δ::LEU2                                              |
| X5624-1A | saw1-K221R-TAP::HIS3 rad59Δ::LEU2                                        |
| SLY5151  | ho HML mat::leu2::hisG hmrΔ3 leu2-3,112 ara3-52 trpl THR4-ura3-3(A205bp)-HOcs-URA3-A ade3::GAL10-HO::NAT |
| X5638-11B| SAW1-TAP::HIS3 apn1Δ::KAN apn2Δ::HIS3                                    |
| X5639-7C | saw1-K221R-TAP::HIS3 apn1Δ::KAN apn2Δ::HIS3                               |
| X5359-9A | SAW1-TAP::HIS3 tdp1Δ::KAN                                                |
| X5360-5A | saw1-K221R-TAP::HIS3 tdp1Δ::KAN                                          |
| X5643-2C | saw1-ΔRBD-TAP::HIS3 tdp1Δ::KAN                                           |
| X5644-8A | saw1-ΔFBD-TAP::HIS3 tdp1Δ::KAN                                           |
| X4965-2D | RAD1-TAP::HIS3                                                           |
| X4965-2B | SAW1-3FLAG::KAN                                                          |
| X4965-2C | RAD1-TAP::HIS3 SAW1-3FLAG::KAN                                           |
| X4967-6B | RAD1-TAP::HIS3 saw1-K221R-3FLAG::KAN                                      |
| X5536-6A | rad55Δ::KAN                                                              |
| X5535-5A | saw1Δ::KAN rad55Δ::KAN                                                   |
| X5530-2D | rad26Δ::KAN                                                              |
| X5529-1B | saw1Δ::KAN rad26Δ::KAN                                                   |
| X5532-11A| rad16Δ::KAN                                                              |
| X5531-10C| saw1Δ::KAN rad16Δ::KAN                                                   |
| X5559-3B | SAW1-TAP::HIS3 rad55Δ::KAN                                               |
| X5536-8D | saw1-K221R-TAP::HIS3 rad55Δ::KAN                                         |
| X5561-1D | SAW1-TAP::HIS3 rad26Δ::KAN                                               |
| X5530-2A | saw1-K221R-TAP::HIS3 rad26Δ::KAN                                         |
| Strain    | Genotype                        |
|----------|---------------------------------|
| X5557-1C | SAW1-TAP::HIS3 rad16Δ::KAN      |
| X5532-10B| saw1-K221R-TAP::HIS3 rad16Δ::KAN|
| X5900-3C | slx1Δ::KAN                      |
| X5899-1B | saw1Δ::KAN slx1Δ::KAN           |
| X5881-3A | slx4Δ::KAN                      |
| X5881-3C | saw1Δ::KAN slx4Δ::KAN           |

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.