DNA Damage Tolerance Pathway Choice Through Uls1 Modulation of Srs2 SUMOylation in *Saccharomyces cerevisiae*

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ABSTRACT DNA damage tolerance and homologous recombination pathways function to bypass replication-blocking lesions and ensure completion of DNA replication. However, inappropriate activation of these pathways may lead to increased mutagenesis or formation of deleterious recombination intermediates, often leading to cell death or cancer formation in higher organisms. Post-translational modifications of PCNA regulate the choice of repair pathways at replication forks. Its monoubiquitination favors translesion synthesis, while polyubiquitination stimulates template switching. Srs2 helicase binds to small ubiquitin-related modifier (SUMO)-modified PCNA to suppress a subset of Rad51-dependent homologous recombination. Conversely, SUMOylation of Srs2 attenuates its interaction with PCNA. Sgs1 helicase and Mus81 endonuclease are crucial for disentanglement of repair intermediates at the replication fork. Deletion of both genes is lethal and can be rescued by inactivation of Rad51-dependent homologous recombination. Here we show that *Saccharomyces cerevisiae* Uls1, a member of the Swi2/Snf2 family of ATPases and a SUMO-targeted ubiquitin ligase, physically interacts with both PCNA and Srs2, and promotes Srs2 binding to PCNA by downregulating Srs2-SUMO levels at replication forks. We also identify deletion of *ULS1* as a suppressor of *mus81Δ sgs1Δ* synthetic lethality and hypothesize that *uls1Δ* mutation results in a partial inactivation of the homologous recombination pathway, detrimental in cells devoid of both Sgs1 and Mus81. We thus propose that Uls1 contributes to the pathway where intermediates generated at replication forks are dismantled by Srs2 bound to SUMO-PCNA. Upon *ULS1* deletion, accumulating Srs2-SUMO—unable to bind PCNA—takes part in an alternative PCNA-independent recombination repair salvage pathway(s).

KEYWORDS DNA damage tolerance; replication stress; SUMO; Srs2; PCNA

ACCURATE duplication of chromosomes is critical for the preservation of genetic information and cell survival. Yet, replication fork progression can be hindered not only by a number of natural physical obstacles in the genome, limiting nucleotides or proteins required for DNA synthesis, but also by spontaneous or induced DNA lesions that escaped repair, resulting in replication stress (Zeman and Cimprich 2013). DNA damage leading to replication fork arrest and single-

strand gap formation opposite the lesion can be temporarily bypassed by several mechanisms referred to as DNA-damage-tolerance (DDT) pathways, dependent on the Rad6-Rad18 complex, and also by Rad51-dependent homologous recombination (HR) when the former fail (Lehner and Jinks-Robertson 2014) (Supplemental Material, Figure S1 in File S1). DDT can be divided into the error-prone mode which involves the recruitment of translesion synthesis (TLS) polymerases that are able to directly replicate across the lesion, often at the cost of fidelity; and the error-free mode which is dependent on Rad5, where template switching occurs, and allows the use of an undamaged chromatid as a template for lesion bypass, reviewed in Sale (2012). The exact mechanism of template switching is unknown, but it has been suggested that it also requires Rad51-dependent HR (Branzei *et al.*

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2008; Vanoli *et al.* 2010; Minca and Kowalski 2011). This demonstrates the importance of both DDT- and HR-mediated pathways in coping with various forms of replication stress, although their relative contributions and interactions have not been well established (Unk *et al.* 2010; Sale 2012; Symington *et al.* 2014).

DDT pathways are regulated by post-translational modifications of proliferating cell nuclear antigen (PCNA) which forms a homotrimeric ring around DNA and serves as a binding platform for DNA polymerases and other replication-associated proteins (Moldovan *et al.* 2007) (Figure S1 in File S1). Formation of single-strand DNA (ssDNA) gaps coated by replication protein A at the stalled replication fork recruits the Rad6-Rad18 E2-E3 ubiquitin ligase complex that promotes monoubiquitination of PCNA at highly conserved Lys164 (Hoegge *et al.* 2002; Davies *et al.* 2008). This modification creates a binding site for TLS polymerases (Bienko *et al.* 2005). The error-free mode of DDT is activated when the monoubiquitinated PCNA is further polyubiquitinated by the E2 heterodimer Ubc13-Mms2 and the E3 ubiquitin ligase Rad5 (Hofmann and Pickart 1999; Ulrich and Jentsch 2000; Hoegge *et al.* 2002). Additionally, during unperturbed replication, the small ubiquitin-related modifier (SUMO) (Smt3 in yeast) is attached to PCNA predominantly at Lys164 by SUMO ligase Siz1, and also at Lys127 by Siz1 and Siz2 (Hoegge *et al.* 2002; Parker *et al.* 2008). It was reported that PCNA SUMOylation mediates the recruitment of the Srs2 helicase to the replication fork, leading to prevention of a subset of HR repair. This HR inhibition by Srs2 is toxic in *rad18* and *rad5* mutants (Papouli *et al.* 2005; Pfander *et al.* 2005), confirming the role of both HR and DDT in the replication stress response. On the other hand, Srs2-PCNA interaction was also demonstrated to limit the length of DNA repair synthesis and thus favor the HR synthesis-dependent strand annealing pathway (SDSA) (Burkovics *et al.* 2013; Miura *et al.* 2013). Importantly, Srs2 was also shown to have the ability to dismantle Rad51 nucleofilaments and to inhibit Rad51-driven DNA strand exchange (Krejci *et al.* 2003; Veaute *et al.* 2003). The Srs2 helicase itself can undergo SUMOylation, which disfavors its binding to SUMO-PCNA but is important for its interaction with Rad51 and other recombination factors (Kolesar *et al.* 2012, 2016). Therefore, modulation of the equilibrium between unmodified and modified forms of Srs2 plays a role in the choice of pathway used to deal with replication stress.

Perturbations in replication fork progression can lead to fork collapse and double-strand break (DSB) formation. Srs2 promotes their repair by SDSA, yielding noncrossing over (non-CO) products and thus limiting loss of heterozygosity events in mitotic cells (Ira *et al.* 2003; Niu and Klein 2016). Nonetheless, D-loops formed after Rad51 recombinase-dependent strand invasion might be stabilized and extended until a second end of a DNA break is captured. Most resulting joint molecules are single or double Holliday junctions (HJs). Double HJs are dissolved by the complex

consisting of the Sgs1 helicase, the DNA topoisomerase Top3, and the stimulatory protein Rmi1 (Gangloff *et al.* 1994; Mullen *et al.* 2005; Cejka *et al.* 2010) resulting in non-CO recombination products. Recombination intermediates that escaped dissolution by the Sgs1-Top3-Rmi1 complex in S phase or were converted into single HJs are resolved by the Mus81-Mms4 endonuclease in G₂/M leading exclusively to CO products (Matos *et al.* 2013; Szakal and Branzei 2013). Any remaining joint molecules are removed by the Yen1 endonuclease during anaphase to complete the repair and disconnect the sister chromatids before chromosome segregation in mitosis (Blanco *et al.* 2014), yielding both CO and non-CO products (Figure S1 in File S1). Single HJ can also be processed by Srs2 (Mitchel *et al.* 2013), demonstrating that Srs2 and Sgs1 can jointly regulate non-CO formation either by promoting the SDSA pathway or by processing HJ-type intermediates. The significance of these alternative pathways preventing the accumulation of toxic recombination intermediates, which arise during replication stress, is manifested by synthetic lethality of *sgs1Δ srs2Δ* and *mus81Δ sgs1Δ* double mutations that can be rescued by deletion of *RAD51* (Mullen *et al.* 2001; Fabre *et al.* 2002).

Uls1 (DIS1/RIS1), due to the presence of the C-terminal Swi2/Snf2-related DNA translocase domain, was implied to play a role in DNA repair and chromatin remodeling. Uls1 also possesses four SUMO-interacting motifs (SIMs) in its N-terminal part and a C-terminal RING domain, a signature structure for ubiquitin ligases. Uls1 interacts with SUMO and Ubc4, the E2 ubiquitin-conjugating enzyme, while *uls1Δ* cells accumulate high-molecular-weight SUMO conjugates, which led to its classification as a SUMO-targeted ubiquitin ligase (STUBL) (Uzunova *et al.* 2007). Additionally, Uls1 was proposed to function in nonhomologous end joining inhibition by dislodging poly-SUMOylated Rap1 molecules from telomeres (Lescasse *et al.* 2013). However, enzymatic activity of Uls1 has never been biochemically demonstrated and little is known about its targets.

We have shown that Uls1 is involved in the replication stress response and its presence is detrimental in the *sgs1Δ* mutant (Cal-Bąkowska *et al.* 2011; Kramarz *et al.* 2014). Importantly, roles of Uls1 in DNA metabolism require an ATP-dependent translocase activity, RING domain, and SIMs (Cal-Bąkowska *et al.* 2011; Chi *et al.* 2011; Lescasse *et al.* 2013; Tan *et al.* 2013; Kramarz *et al.* 2014). It can therefore be hypothesized that Uls1 may regulate DNA-repair-pathway choice at the sites of replication-associated DNA damage by modifying SUMOylation status and thus the abundance and/or activities of its target proteins.

Here we identify deletion of *ULS1* as a suppressor of *mus81Δ sgs1Δ* synthetic lethality and suggest that Uls1 assists in a Rad51-dependent pathway detrimental in this mutant. We also demonstrate that Uls1 downregulates the level of Srs2 SUMOylation, favoring Srs2 interaction with PCNA, and thus promoting the PCNA-Srs2-dependent DNA damage tolerance pathway. We propose that this activity might contribute to Uls1 toxicity in mutants devoid of the Sgs1 helicase.

Materials and Methods

Yeast strains, plasmids, and growth conditions

Strains and plasmids used in this study are listed in Tables S1 and S2 in [File S1](#), respectively. Gene deletion or tagging was performed using either a PCR-based replacement method or by genetic crossing of relevant mutants followed by tetrad dissection. Doubling time measurements were performed as previously described (Bernstein *et al.* 2009). To assess the sensitivity of selected mutants to hydroxyurea (HU) or methyl methanesulfonate (MMS), midlog-phase cells were serially diluted and spotted onto solid media containing various concentrations of DNA-damaging agents. Plates were photographed after 3 days of incubation at 30°. To synchronize cells in G₁, midlog cultures were incubated for 2 hr in the presence 5 μ M α -factor. Subsequently, pheromone was removed by extensive washing with water and cells were released into medium containing the appropriate genotoxic agent.

Viability counts

To determine the effect of *GALS-HA-ULS1* expression in the *mus81 Δ sgs1 Δ* background on cell viability, the triple mutant was cultured in YPD medium to an OD₆₀₀ of 0.8. After extensive washing, one half of the culture was transferred to fresh YPD and the second half was resuspended in YPGal medium. After 4 hr incubation, cultures were diluted to an OD₆₀₀ of 0.8 in the same medium. At the indicated time points, samples were washed, diluted, and plated onto YPD medium to determine the number of viable cells able to form colonies. The numbers of viable cells were normalized by their values at time zero.

Yeast two-hybrid assay

Gal4-based Matchmaker Two-Hybrid System 3 (Clontech) was used to study the protein interactions according to manufacturer's instructions. The two-hybrid constructs used in this study were described in Parker and Ulrich (2012), Tan *et al.* (2013), and Kolesar *et al.* (2016); except for the following truncated versions of *ULS1* designed for this work: pGADT7-Uls1_N (2-878), pGADT7-Uls1_C (879-1619), pGBKT7-Uls1_N (2-878), and pGBKT7-Uls1_C (879-1619).

Analysis of whole-protein extracts

Protein extracts were prepared by the trichloroacetic acid (TCA) method. Briefly, logarithmic cells of indicated strains were harvested and lysed with lysis buffer (2 M NaOH, 7% β -mercaptoethanol). Total protein was precipitated by adding 50% TCA, pellet was washed with 1 M Tris at pH 8 and resuspended in 2 $\times$ Laemmli buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 2% β -mercaptoethanol). Obtained samples were analyzed by SDS-PAGE and Western blotting using anti-Srs2 (sc-11989; Santa Cruz) or anti-HA (12CA5; Roche). Blotted membranes were stained with the Ponceau S reagent (Sigma-Aldrich) to detect total proteins.

Detection of SUMOylated Srs2

Cells transformed with pWJ1509 (*pCUP1-SRS2*) or pAM19 (*pCUP1-srs2-R1*) (León Ortiz *et al.* 2011) were grown to mid-

log phase followed by 4 hr incubation in the presence of 200 μ M CuSO₄ to induce *SRS2* gene expression. Cell pellets were collected, washed once with 1 ml of 20% TCA, and frozen. For immunoprecipitation (IP) in denaturing conditions, cells were resuspended in 1 ml of 20% TCA and disrupted by bead beating. Precipitates were recovered by centrifugation (10 min, 13,000 rpm, 4°). Denatured proteins in pellets were resuspended in 2 $\times$ Laemmli buffer and boiled for 5 min. Then, 10 μ l of the resulting protein extracts was used as a loading control for the IP reaction. For each IP, 100 μ l of protein extract was diluted with 400 μ l of IP buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% NP-40) and 40 μ l of anti-Srs2 (sc-11989; Santa Cruz) antibody was added. The IP reaction was allowed to continue overnight at 4° on a rotator wheel. The negative control for each IP was the diluted protein extract with IP buffer without anti-Srs2 antibody. The next day, prewashed Protein G Magnetic Beads (Dynabeads; Invitrogen, Carlsbad, CA) were added for additional 2 hr at 4°. Beads were washed three times with IP buffer and bound proteins were eluted with 20 μ l of 2 $\times$ Laemmli buffer for 10 min at 65°. The eluted proteins were resolved on Mini-PROTEAN TGX Precast Gel 4–15% (Bio-Rad, Hercules, CA) and Western blot using anti-Srs2 (sc-11989; Santa Cruz) antibody was performed. After visualizing Srs2 protein, the membrane was stripped with 0.4 M NaOH and probed with anti-SUMO antibody (200-401-428; Rockland) to detect the SUMOylated forms of Srs2 protein.

Quantification of Srs2 and Srs2-SUMO levels

To quantify the levels of Srs2 in analyzed strains, the signal corresponding to Srs2 and its SUMOylated forms was measured and compared with the Ponceau S signal at the respective molecular weight or immunoprecipitated Srs2 band using Image Lab (Bio-Rad). In the analysis of protein stability by cycloheximide (CHX) chase assay, the intensities of Srs2 bands vs. those of G-6-PDH bands were quantified with Image Lab (Bio-Rad). Statistical significance of observed differences was examined by *t*-test calculation of the *P*-values from independent experiments with GraphPad Software (QuickCalcs).

Analysis of protein stability

Yeast cells were synchronized in G₁ phase and released into YPD containing 0.03% MMS and 50 μ g/ml CHX. Samples were collected at indicated time points and disrupted by bead beating in 20% TCA as described above. Once lysed, denatured proteins were resuspended in SDS-loading Sample Buffer (80 mM Tris-HCl, pH 8.0, 3.5% SDS, 15% glycerol, 100 mM DTT, 8 mM EDTA, 1 mM PMSF) and incubated for 20 min at 42°. Next, protein extracts were boiled for 4 min and subjected to SDS-PAGE and Western blotting with the anti-Srs2 antibody (sc-11989; Santa Cruz) and anti-G-6-PDH (A9521-1VL; Sigma-Aldrich) as a loading control.

Co-IP

Cells were grown to midlog phase, harvested, and frozen at –80°. Co-IP using the rabbit polyclonal anti-PCNA (Pol30) antibody

(Zhang *et al.* 2000) was performed with lysis buffer L1 (50 mM Tris at pH 8.0, 100 mM NaCl, 1 mM EDTA, 5 mM MgCl₂, 1 mM DTT, 10% glycerol, 0.5 mM PMSF). Co-IP using anti-HA (12CA5; Roche) antibodies was performed with lysis buffer L2 (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 10% glycerol, 1% NP-40, 2 mM EDTA). In both cases, cells were resuspended in 700 µl of pre-cooled lysis buffer L1 or L2 supplemented with cOmplete Protease Inhibitor Cocktail (Roche) and 1 mM PMSF. Then cells were disrupted by bead beating at 4°. The appropriate antibody was added to resulting total extracts and the IP reaction was allowed to continue overnight at 4° on a rotator wheel. The negative control in each case was total extract without addition of the respective antibody. The next day, prewashed Protein G Magnetic Beads (Dynabeads; Invitrogen) were added to each reaction for an additional 2 hr. Then, beads were washed three times with an adequate lysis buffer and bound proteins were eluted with 20 µl of 2× Laemmli buffer for 10 min at 65°. The eluted proteins were resolved on Mini-PROTEAN TGX Precast Gel 4–15% (Bio-Rad) and transferred onto nitrocellulose membrane. After Ponceau S staining, the membrane was cut into fragments corresponding to the size of analyzed proteins. Each fragment was then immunoblotted with the appropriate primary antibody (anti-PCNA, anti-Srs2, or anti-HA).

Data availability

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article. Plasmids and strains are available upon request.

Results

Uls1 presence contributes to the severe synthetic growth defect of the double mutant devoid of *Mus81* endonuclease and *Sgs1* helicase

We have previously shown that *Uls1* has a role in dealing with perturbations in DNA replication that is independent of both *Mus81* and *Yen1* endonucleases, and that deletion of *ULS1* attenuates the replication stress-related defects of the *sgs1Δ* mutant in a *Rad51* recombinase and *Rad18*-, *Rad5*-dependent manner (Kramarz *et al.* 2014).

It was thus interesting to examine the effect of *ULS1* deletion on the *mus81Δ sgs1Δ* double mutant. We found upon sporulation of the heterozygous diploid that most spores of the *sgs1Δ mus81Δ uls1Δ* genotype were viable, although slow growing (Figure 1A). To confirm that the observed rescue of a *sgs1Δ mus81Δ* double mutant's severe synthetic growth defect was indeed due to the depletion of *Uls1* protein, we constructed a heterozygous diploid containing *sgs1Δ* and *mus81Δ* as well as the *HA-ULS1* gene under the control of a *GALS* promoter inserted in the endogenous *ULS1* locus. We demonstrated that adding an HA tag does not change *Uls1* activity, since the presence of HA-*Uls1* rescued the increased genotoxin sensitivity of the *mus81Δ GALS-HA-ULS1* mutant (Figure S2 in File S1). In addition, overproduction of HA-*Uls1*

from either *GALS* or the *ADH1* promoter was toxic in the *sgs1Δ* mutant (Figure S2 in File S1) as described before for untagged *Uls1* (Cal-Bąkowska *et al.* 2011). We then analyzed several *mus81Δ sgs1Δ GALS-HA-ULS1* clones on YP medium containing glucose or galactose as a carbon source when the expression of *ULS1* gene was repressed or induced, respectively. We found that the *mus81Δ sgs1Δ GALS-HA-ULS1* mutant cultured on glucose increased its cell numbers nearly eightfold during 24 hr, while shifting the cells to galactose resulted in a decrease in the viable cell numbers to about one fourth of the initial cell count (Figure 1B). This corresponded with the maximum of appearance of the HA-*Uls1* protein after 12 hr induction, as indicated by Western blot (Figure 1C).

It is worth noting that phenotypes of the *mus81Δ sgs1Δ GALS-HA-ULS1* and *mus81Δ sgs1Δ uls1Δ* mutants grown on media containing glucose were indistinguishable and comparable to that of the *mus81Δ sgs1Δ rad51Δ* mutant, a well-known suppressor of the *mus81Δ sgs1Δ* synthetic growth defect (Fabre *et al.* 2002). They all had growth defects, with generation times (180–190 min) twice as long as wild type, and were extremely sensitive to MMS and HU (Figure 1D and data not shown).

Srs2 as a target of *Uls1* activity

The *srs2Δ sgs1Δ* double mutant's synthetic sickness is also rescued by deletion of *RAD51* (Klein 2001; Fabre *et al.* 2002), but here we show that deletion of *ULS1* did not lead to the rescue of the synthetic lethality of a *sgs1Δ srs2Δ* double mutant (Figure S3 in File S1). This raises the possibility that the *Srs2*-dependent pathway might play a role in the alleviation of the replication stress-related defects in *sgs1Δ* cells by deletion of *ULS1*.

Srs2 protein is known to be SUMOylated in a manner largely dependent on the presence of its SIM (Kolesar *et al.* 2012). *Uls1*, as a proposed SUMO-targeted ubiquitin ligase (Uzunova *et al.* 2007; Lescasse *et al.* 2013; Kramarz *et al.* 2014), could affect this post-translational modification and thus influence *Srs2* activity.

To investigate this possibility, we transformed wild type, *uls1Δ*, and a strain overexpressing *ULS1* from a constitutive *ADH1* promoter with plasmids harboring genes for *SRS2* or *srs2-R1*, devoid of a SIM (Le Breton *et al.* 2008; León Ortiz *et al.* 2011), under the control of a copper-induced *CUP1* promoter. We found that in the protein extract from the *uls1Δ* strain overexpressing the *SRS2* gene, *Srs2* protein migrated as a more profuse band of heavier molecular weight (Figure 2A and Figure S4A in File S1). This was not seen for the *uls1Δ* strain overexpressing the *srs2-R1* allele, or for wild type overexpressing *SRS2* or *srs2-R1*, suggesting that deletion of *ULS1* might lead to the accumulation of heavy molecular, possibly SUMOylated, forms of *Srs2*. Moreover, in strains overexpressing *ULS1*, the level of both *Srs2* and *Srs2-R1* was reduced approximately by half (Figure 2A and Figure S4A in File S1). To confirm that the accumulating heavy molecular forms of *Srs2* were indeed SUMOylated, we immunoprecipitated

Figure 1 Deletion of *ULS1* suppresses the synthetic lethality of *mus81Δ sgs1Δ* double mutant. (A) The triple *mus81Δ sgs1Δ uls1Δ* (KK028) mutant is viable but exhibits slow-growth phenotype as determined by tetrad analysis. (B) Decreased survival of *mus81Δ sgs1Δ GALS-HA-ULS1* (KK029) cells after induction of *ULS1* expression. (C) Analysis of HA-Uls1 protein levels. The *mus81Δ sgs1Δ GALS-HA-ULS1* (KK029) mutant was grown in YPD medium to log phase, washed, and resuspended in YPGal medium. Cell samples were taken at the indicated time points for viability determination, protein extraction, and Western blot analysis with anti-HA antibodies. (D) The *mus81Δ sgs1Δ GALS-HA-ULS1* (KK029) cells grown on glucose medium (YPD) exhibit the same phenotype as *mus81Δ sgs1Δ uls1Δ* (KK028). The level of suppression is the same as in *mus81Δ sgs1Δ rad51Δ* (KK030) but all mutants show growth defect compared to wild type (WT) (W303-1A).

proteins from extracts from the transformants described above, using the anti-Srs2 antibody, and analyzed the immunoprecipitates by Western blot with anti-SUMO and anti-Srs2 antibodies. Indeed, deletion of *ULS1* resulted in the accumulation of SUMOylated forms of Srs2 protein, while overproduction of Uls1 led to a decrease in the amount of Srs2-SUMO (Figure 2B compare lane 2 with 4 and 6, and Figure S4B in File S1). The Srs2 SIM has earlier been demonstrated to be required not only for its binding to PCNA, but also for its SUMOylation under unperturbed conditions (Le Breton *et al.* 2008; Armstrong *et al.* 2012; Kolesar *et al.* 2012). Consistently, we observed virtually no Srs2-SUMO conjugates in immunoprecipitates from transformants containing Srs2-R1 protein, devoid of SIM (Figure 2B compare lane 2 with 8, 10, 12; and Figure S4B in File S1).

Srs2 has been shown to interact with Slx5, a second STUbL (Urulangodi *et al.* 2015), so we compared the level of Srs2-SUMO in *slx5Δ* and *uls1Δ* mutants overexpressing *SRS2*. In total protein extract preparations from *slx5Δ* and *uls1Δ* mutants, we could observe a significant increase in the signal corresponding to the Srs2 protein and its heavier, modified forms, compared to wild type (Figure 3A). We probed Srs2 immunoprecipitates from these strains with anti-SUMO antibody and confirmed that these species corresponded to Srs2-SUMO (Figure 3B). Taken together, these data demonstrate that deletion of *SLX5* as well as *ULS1* results in the accumulation of Srs2-SUMO conjugates.

The analysis of protein stability by CHX chase revealed that Slx5-Slx8 STUbL is involved in the regulation of endogenous

Srs2 levels (Urulangodi *et al.* 2015). Since we found, as shown in Figure 2A, that in strains overexpressing *ULS1* the level of Srs2 is reduced, we also examined the stability of the endogenous Srs2 protein by CHX chase in wild type and *uls1Δ* and compared it to the *slx5Δ* mutant. We found that in both mutants the Srs2 protein level was stabilized, while it dropped by about half in wild type (Figure 3C); confirming that Uls1 also mediates Srs2 turnover, similarly to Slx5-Slx8 (Urulangodi *et al.* 2015). However, *SLX5* and *SLX8* have been shown to have negative interactions with *ULS1* (Collins *et al.* 2007) and we also demonstrated that the *uls1Δ slx5Δ* mutant has a stronger growth defect and is more sensitive to HU and MMS than the single *slx5Δ* mutant (Kramarz *et al.* 2014). Additionally, Tan *et al.* (2013) described an antagonistic relationship between *ULS1* and *SLX5*. Moreover, while deletion of *uls1Δ* alleviates *sgs1Δ* sensitivity to MMS and HU (Cali-Bąkowska *et al.* 2011), the *slx5Δ sgs1Δ* double mutant is lethal (Mullen *et al.* 2001). This suggests that Uls1 and Slx5 also have separate functions in the cell.

We were unable to detect a signal from Uls1 on Western blot when it was expressed from its native promoter, so to examine the Uls1-Srs2 interaction we used the *srs2Δ* strain overexpressing HA-*ULS1* and either wild-type *SRS2* or *srs2-R1*. We demonstrated by IP that Uls1 was able to bind to Srs2 even in the absence of SIM in Srs2 (Figure 4A). Using the yeast two-hybrid system, we mapped the Srs2-Uls1 interaction site to the N-terminal fragment of Uls1 and C-terminal part of Srs2 and showed that it was independent of the Srs2 SIM (Figure 4B) in accordance with the IP data. This finding

A

B

Figure 2 Srs2 is a target of Uls1 activity. (A) Uls1 regulates the protein level of Srs2 helicase. Wild type (WT) (W303-1A), *uls1Δ* (MC001), and *ADH1-HA-ULS1* (MC037) strains were transformed with *CUP1-SRS2* (pWJ1509) or *CUP1-srs2-R1* (pAM19) plasmids and grown to logarithmic phase in selective medium followed by 4-hr incubation in the presence of 200 μ M CuSO_4 to induce overexpression of *SRS2* alleles. Then cells were collected for protein extraction and Western blot analysis using anti-Srs2 antibody. The *srs2Δ* mutant (MC010) was included in the analysis as a control of antibody specificity. The signal detected for Srs2 vs. total protein as determined by Ponceau-S staining was quantified for three experiments. (B) SUMOylation status of Srs2 is affected by activity of Uls1. Aliquots of protein extracts obtained in (A) were used for IP reaction with anti-Srs2 antibody. Immunoprecipitates were analyzed by immunoblotting with anti-Srs2 and anti-SUMO antibodies. Srs2-SUMO levels vs. total immunoprecipitated Srs2 from two experiments were quantified. SDs are shown and Student's *t*-test was used to calculate the *P*-value (* 0.01 < *P*-value $\leq$ 0.05, ** 0.001 < *P*-value $\leq$ 0.01).

Figure 3 Comparison of the effect of *SLX5* and *ULS1* deletion on the accumulation of SUMOylated Srs2. (A) Wild type (WT) (W303-1A), *uls1Δ* (MC001), and *slx5Δ* (KK013) strains were transformed with *CUP1-SRS2* (pWJ1509) and after overexpression of *SRS2* (as in Figure 2), protein extracts were isolated and Western blot analysis with anti-Srs2 antibody was performed. The signal detected for Srs2 vs. total protein as determined by Ponceau-S staining was quantified for two experiments. (B) IP of Srs2 from extracts obtained in (A) and visualization of SUMOylated Srs2 using anti-Srs2 and anti-SUMO antibody. Srs2-SUMO levels vs. total immunoprecipitated Srs2 from two experiments were quantified. (C) *Uls1* and *Slx5* mediate turnover of the Srs2 protein. Endogenous Srs2 level was analyzed by CHX chase. Wild type (W303-1A), *uls1Δ* (MC001), and *slx5Δ* (KK013) were synchronized in G₁ and released into YPD medium containing 0.03% MMS and 50 μg/ml CHX. At the indicated time points, cells were collected for protein extraction followed by Western blotting with the anti-Srs2 antibody. Level of G-6-PDH was used as a loading control. The *srs2Δ* mutant (MC010) was included in the analysis as a control of antibody specificity. The percentage values of relative Srs2 level vs. G-6-PDH, acquired for two experiments, are plotted. SDs are shown and Student's *t*-test was used to calculate the *P*-value (* 0.01 < *P*-value ≤ 0.05).

is consistent with *Uls1*'s ability to regulate *Srs2* protein levels independently of *Srs2*'s SUMOylation status (Figure 2A) and reports in the literature (Xie *et al.* 2010; Abed *et al.* 2011) showing that STUBs can also bind to their substrates through SUMO-independent interactions. The fact that *Uls1* can bind *Srs2* in the absence of the *Srs2* SIM might indicate that the *Uls1*-*Srs2* interaction is independent from *Srs2*-PCNA binding and also from *Srs2* SUMOylation.

We thus identified *Srs2* as a possible *in vivo* physiological target for *Uls1*.

Deletion of *ULS1* compromises *Srs2*-PCNA interaction

Previous work has demonstrated that overexpression of *SRS2* has a negative effect on growth in wild type and even more so in many mutant backgrounds (Mankouri *et al.* 2002; León Ortiz *et al.* 2011). It has also been shown that an increased abundance of the helicase dead mutant form of *Srs2* is more toxic than the wild-type protein, while overproduction of mutant forms unable to interact with PCNA, such as *Srs2*-R1, is not toxic in wild type and the vast majority of tested mutants (León Ortiz *et al.* 2011).

Using strains with *HA-SRS2* and *HA-srs2-R1* genes expressed from a methionine-repressible promoter introduced into an *SRS2*-endogenous locus, we examined the effect of *ULS1* deletion on *Srs2* overproduction toxicity. We found that when *HA-SRS2* is overexpressed, the wild-type cells were much more sensitive to HU and MMS than *uls1Δ* mutant cells. Overexpression of *HA-srs2-R1*, however, was less toxic and conferred the similar degree of HU and MMS sensitivity in both wild type and the *uls1Δ* mutant (Figure 5A). Since it has been shown that SUMOylation decreases the ability of *Srs2* to bind PCNA (Kolesar *et al.* 2012), we propose that lower toxicity of *SRS2* overexpression in the *uls1Δ* mutant results from accumulation of SUMOylated forms of *Srs2* in this mutant background, as shown in Figure 2B and Figure 3B. This would compromise *Srs2* binding to PCNA and reduce the toxicity of *SRS2* overexpression. These data suggest a physiological function for *Uls1* in regulating *Srs2* binding to PCNA through modification of *Srs2*'s SUMOylation status.

We thus assayed the influence of *Uls1* on *Srs2* interaction with PCNA more directly. Interestingly, we found that *Uls1* itself immunoprecipitated with PCNA and this interaction

Figure 4 Uls1 interacts with Srs2. (A) Protein extracts prepared from *srs2Δ ADH1-HA-ULS1* (KK032) cells overexpressing either *SRS2* (from pWJ1509 plasmid) or *srs2-R1* (from pAM19 plasmid) were immunoprecipitated with anti-HA antibody, followed by Western blot analysis with anti-HA and anti-Srs2 antibodies. The negative controls for IP reactions are marked as “–Ab.” (B) The N-terminal part (2–878) of Uls1 (pGBKT7-Uls1_N plasmid) is responsible for interaction with C-terminal part (783–1174) of Srs2 (pLK165 plasmid) and Srs2ΔSIM (pLK1002 plasmid) variant as shown by yeast two-hybrid assay (in AH109 strain).

was independent of the presence of Srs2 (Figure 5B, galactose). Using the yeast two-hybrid system we mapped the PCNA-Uls1 interaction site to the N-terminal fragment of Uls1 and showed that Uls1 SIMs were not critical for Uls1 binding to PCNA (Pol30). We also demonstrated that the Uls1-PCNA interaction did not require PCNA SUMOylation, as the Pol30RR, lacking SUMOylation sites, and wild-type Pol30 were indistinguishable in our assay (Figure 5C).

Under standard growth conditions without genotoxin treatment, we could immunoprecipitate Srs2 with PCNA both in the wild-type strain and in the *GALS-HA-ULS1* strain grown on galactose, but we were unable to immunoprecipitate Srs2 with PCNA in the *GALS-HA-ULS1* strain grown on glucose when expression of *ULS1* was blocked (Figure 5B, glucose). These results show that in the absence of Uls1, binding of Srs2 to PCNA is compromised. It is well documented (Le Breton *et al.* 2008; Kolesar *et al.* 2012) that Srs2 SUMOylation negatively affects its PCNA binding and, since we show that deletion of *ULS1* leads to the accumulation of Srs2-SUMO, it follows that the Srs2-PCNA interaction would be attenuated in this mutant. Overall our results suggest that Uls1 can regulate Srs2's ability to bind PCNA by modifying Srs2's SUMOylation status.

The role of Srs2 modifications in the suppression of the *sgs1Δ* mutant phenotype by deletion of *ULS1*

Interestingly, lack of Srs2 SUMOylation and its binding to PCNA are not responsible for the severe growth defect observed in the *sgs1Δ srs2Δ* strain (Le Breton *et al.* 2008;

Kolesar *et al.* 2016). We constructed *sgs1Δ HA-srs2-R1* and *sgs1Δ uls1Δ HA-srs2-R1* mutants and found that the *HA-srs2-R1* mutation diminished the extent of rescue of *sgs1Δ* mutant's HU and MMS sensitivity by deletion of *ULS1*, even though it had no effect on the *sgs1Δ* mutant (Figure 6A). This raises the possibility that not only compromising the Srs2-PCNA interaction but also accumulation of SUMOylated Srs2 may be important for the *sgs1Δ uls1Δ* phenotype. To test that assumption, we constructed *sgs1Δ srs2-3KR* and *sgs1Δ uls1Δ srs2-3KR* mutants, containing Srs2 with mutated SUMOylation sites but proficient in PCNA binding. We found that the *srs2-3KR* mutation also reversed the rescue of *sgs1Δ* mutant's HU and MMS sensitivity by deletion of *ULS1* (Figure 6B), and the absence of Srs2 SUMOylation did not lead to the synthetic growth defect in the *sgs1Δ* background.

We thus propose that Srs2-SUMO is important for genome stability and may play a role in the additional salvage pathway activated in the absence of Uls1.

Rad51-dependent pathway promoted by Uls1 is responsible for the synthetic growth defect in the *sgs1Δ mus81Δ* double mutant

It has been shown before that elimination of HR suppresses the lethality of *mus81Δ sgs1Δ* cells (Fabre *et al.* 2002). We show that deletion of *ULS1* suppresses *mus81Δ sgs1Δ* synthetic lethality to the same degree as deletion of *RAD51*. Moreover, the quadruple mutant, *mus81Δ sgs1Δ rad51Δ GALS-HA-ULS1*, has a similar phenotype (Figure 7A), suggesting that Uls1 assists in a Rad51-dependent pathway that becomes toxic in cells devoid of both Sgs1 and Mus81.

Attenuation of the *sgs1Δ* mutant phenotype by deletion of *ULS1* requires the presence of Rad51 and Rad5 (Cal-Bąkowska *et al.* 2011; Kramarz *et al.* 2014), so we examined the effect of *RAD5* deletion in a *mus81Δ sgs1Δ* background. We found that deletion of *RAD5* slightly rescued the severe synthetic growth defect of the *mus81Δ sgs1Δ* double mutant, suggesting that some aspect of Rad5 activity may be detrimental in cells lacking Sgs1 and Mus81. Interestingly, deletion of *RAD5* reversed the beneficial effect of both *RAD51* as well as *ULS1* deletion on the phenotype of the *mus81Δ sgs1Δ* mutant (Figure 7A). These results suggest that a functional Rad5-dependent subpathway is required for the observed rescue of synthetic lethality of the *mus81Δ sgs1Δ* double mutant in the *rad51Δ* and *uls1Δ* mutant backgrounds.

Since the presence of *ULS1* is toxic in the *mus81Δ sgs1Δ* mutant background, similarly to that of *RAD51*, we could hypothesize that the *uls1Δ* mutation results in a partial inactivation of the Rad51-dependent pathway. To corroborate this assumption, we examined the interaction of *uls1* with other mutants in two systems that are established as being indicative of attenuation of HR pathways. First, it has been shown that deletion of *SRS2* rescues *rad18Δ* and *rad5Δ* mutants' slow growth as well as their UV and MMS sensitivity, in a manner dependent on the presence of Rad51, suggesting a requirement for HR (Friedl *et al.* 2001; Papouli *et al.* 2005). If Uls1 is involved in HR, its depletion should not lead to the

Figure 5 The role of Uls1 in Srs2-PCNA interaction. (A) Toxic effects of *SRS2* overexpression are alleviated by deletion of *ULS1*. Wild type (WT) (W303-1A), *HA-SRS2* (CY2715), *HA-SRS2 uls1*Δ (SM001), *HA-srs2-R1* (D511-4D), *HA-srs2-R1 uls1*Δ (SM002), *MET25-HA-SRS2* (SM003), *MET25-HA-SRS2 uls1*Δ (SM004), *MET25-HA-srs2-R1* (SM005), *MET25-HA-srs2-R1 uls1*Δ (SM006), and *srs2*Δ (MC010) were spotted onto minimal medium without methionine to induce overexpression of *MET25-HA-SRS2* or *MET25-HA-srs2-R1*. Plates were photographed after 3 days of incubation at 30°. (B) Uls1 interacts with PCNA and promotes Srs2 binding to PCNA. Logarithmically growing cells of wild type (W303-1A), *GALS-HA-ULS1* (MC038), and *srs2*Δ *GALS-HA-ULS1* (KK031) were grown on either YPD medium to repress, or YPGal medium to induce, *ULS1* overexpression. Aliquots of protein preparation from total cells extracts (TE) and immunoprecipitates with anti-PCNA antibody were analyzed by Western blot with anti-HA, anti-PCNA, and anti-Srs2 antibodies. The negative controls for IP reactions are marked as “-Ab.” “M” stands for molecular weight marker. (C) The N-terminal part (2–878) of Uls1 (pGADT7-*Uls1*_N plasmid) interacts with PCNA (Pol30, #618 plasmid) and PCNA K127/164R (Pol30RR, #1040 plasmid) as shown by yeast two-hybrid assay (in AH109 strain).

rescue of *rad18*Δ and *rad5*Δ mutants’ phenotypes. In accordance with that assumption, we demonstrated that deletion of *ULS1* had no effect on *rad18*Δ and *rad5*Δ mutant phenotypes and even slightly reversed the rescue observed in the *rad18*Δ *srs2*Δ and *rad5*Δ *srs2*Δ mutants (Figure 7B), as has been demonstrated before for *Rad51* (Papouli *et al.* 2005). This implies that deletion of *ULS1* partially impairs the *Rad51*-mediated HR pathway, making the cells more reliant on the *Rad18*-*Rad5*-dependent one. Second, cells lacking a

structure-selective flap endonuclease, *Rad27*, depend on HR for viability (Symington 1998). We found that deletion of *ULS1* in the *rad27*Δ background led to severe growth defect of double mutants, which is consistent with the role of *Uls1* in the process of HR (Figure 7C).

We thus propose that *Uls1* contributes to the pathway where intermediates generated at replication forks by *Rad51* (and *Rad5*) are dismantled by *Srs2* bound to SUMO-PCNA. Upon *ULS1* deletion, accumulating *Srs2*-SUMO,

Figure 6 Srs2 involvement in the suppression of *sgs1Δ* mutant phenotype by deletion of *ULS1*. (A) The presence of *srs2-R1* decreased synthetic rescue observed in *sgs1Δ uls1Δ*. *sgs1Δ HA-SRS2* (SM007), *sgs1Δ uls1Δ HA-SRS2* (SM008), *sgs1Δ HA-srs2-R1* (SM009), and *sgs1Δ uls1Δ HA-srs2-R1* (SM010) were spotted onto YPD plates in the presence or absence of genotoxic agents. (B) Synthetic rescue phenotype visible in *sgs1Δ uls1Δ* depends on the presence of SUMOylated form of Srs2. *srs2-3KR* (SS149-15D) was introduced into *sgs1Δ* (MC012) and *sgs1Δ uls1Δ* (MC013), and growth of resulting mutants *sgs1Δ srs2-3KR* (KK033) and *sgs1Δ uls1Δ srs2-3KR* (KK034) on plates with HU and MMS was examined.

unable to bind PCNA, takes part in an alternative recombination repair pathway(s) (Figure 8) involving other recombination factors (Kolesar *et al.* 2016) and possibly also Rad5.

Discussion

We report that deletion of *ULS1* suppresses the severe growth defect of the *mus81Δ sgs1Δ* double mutant similarly to deletion of *RAD51*, suggesting that Uls1 may be involved in the same pathway as Rad51, leading to the accumulation of recombination intermediates that are toxic in cells devoid of both Sgs1 and Mus81. We also demonstrate that deletion of *ULS1* does not lead to the rescue of the synthetic lethality of a *sgs1Δ srs2Δ* double mutant. Therefore we hypothesized that the Srs2-dependent pathway might play a role in the suppres-

sion of the replication stress-related defects in *sgs1Δ* and *mus81Δ sgs1Δ* cells by deletion of *ULS1*.

Consequently, we found that in the absence of Uls1, Srs2-SUMO conjugates accumulate and the protein is stabilized, as is also observed in the *slx5Δ* mutant (Urulangodi *et al.* 2015). Uls1 can bind to both Srs2 and PCNA, but interestingly, we were unable to immunoprecipitate Srs2 with PCNA in the *uls1Δ* mutant, suggesting that in this mutant Srs2-PCNA interaction was attenuated. This is consistent with previous reports (Le Breton *et al.* 2008; Kolesar *et al.* 2012) that Srs2-SUMO is compromised in binding to PCNA. This result suggests that Uls1, by limiting the pool of SUMOylated Srs2, may promote the Srs2-PCNA interaction.

It has been shown before that the ability of Srs2 to bind PCNA is responsible for the toxicity of Srs2 overproduction in most mutant backgrounds, and that overexpression of *srs2-R1* (unable to bind PCNA and not SUMOylated) is not detrimental (León Ortiz *et al.* 2011). Consistently with our biochemical findings, we demonstrate that deletion of *ULS1* reduces the toxicity of *SRS2* overexpression in wild type. This result supports our conjecture that the form of Srs2 abundant in the absence of Uls1 is indeed defective in PCNA binding. We thus propose Srs2 to be a physiological target for Uls1.

Taken together, our data imply that during the replication stress response, Uls1 influences Srs2- and Sgs1-dependent pathways. In *S. cerevisiae* cells grown under unperturbed conditions, replication forks can encounter various obstacles, leading to fork stalling, collapse, or even DSB formation. In response to such mild replication stress, Rad51- and Rad5-dependent, error-free postreplication pathways like SDSA and the PCNA polyubiquitination-dependent template switch are activated (Branzei *et al.* 2008; Burkovich *et al.* 2013; Miura *et al.* 2013). Additionally, PCNA-SUMO mediates the

Figure 7 Uls1 acts on Rad51-dependent pathway. (A) The effect of depletion of Uls1, Rad5, and Rad51 on suppression of synthetic lethality of *mus81Δ sgs1Δ* double mutant. Wild type (WT) (W303-1A), *mus81Δ sgs1Δ rad51Δ* (KK030), *mus81Δ sgs1Δ rad5Δ* (KK035), *mus81Δ sgs1Δ GALS-HA-ULS1* (KK029), *mus81Δ sgs1Δ rad51Δ rad5Δ* (KK036), *mus81Δ sgs1Δ rad51Δ GALS-HA-ULS1* (KK037), *mus81Δ sgs1Δ rad5Δ GALS-HA-ULS1* (KK038), and *mus81Δ sgs1Δ rad51Δ rad5Δ GALS-HA-ULS1* (KK039) were spotted onto YPD plates in the presence or absence of genotoxins. (B) Comparison of suppression of *rad18Δ* and *rad5Δ* DNA-damage sensitivity in cells devoid of Srs2 and Uls1. Indicated strains *rad5Δ* (KK040), *rad5Δ uls1Δ* (KK041), *rad5Δ srs2Δ* (KK042), *rad5Δ srs2Δ uls1Δ* (KK043), *rad18Δ*

(MC035), *rad18Δ uls1Δ* (MC036), *rad18Δ srs2Δ* (KK044), and *rad18Δ srs2Δ uls1Δ* (KK045) were spotted onto YPD medium containing DNA damaging agents. (C) *uls1Δ* exhibits synthetic growth defect with *rad27Δ*. Heterozygous diploid strains for both mutations were sporulated and tetrads were dissected to examine growth of indicated haploid strains *rad27Δ* (KK046) and *rad27Δ uls1Δ* (KK047).

Figure 8 Model for the role of Uls1 in replication stress response. An orange oval with S denotes SUMO. See the text for details.

recruitment of the *Srs2* helicase to the replication fork (Papouli *et al.* 2005; Pfander *et al.* 2005). In this way, recombination at the replication fork is downregulated. Genetic data from *S. cerevisiae* suggest that *Sgs1* is involved in promoting SDSA (Mitchel *et al.* 2013), although its exact activity is uncertain. *Sgs1* has also been implicated in a dissolution of *Rad5*-mediated template switch intermediates (Branzei *et al.* 2008) and reversion of regressed replication forks (Lo *et al.* 2006). We demonstrate that *Uls1* promotes *Srs2* binding to PCNA, presumably by decreasing the amount of *Srs2*-SUMO at replication forks. So we propose a model in which *Uls1* acts by channeling *Rad51*- and *Rad5*-generated intermediates into the PCNA-*Srs2*-dependent pathway (Figure 8). We infer that this is the source of *Uls1* toxicity in *sgs1Δ* as well as in the *mus81Δ sgs1Δ* mutant.

In the *uls1Δ* mutant, the *Srs2*-SUMO accumulates and is unable to bind PCNA. We have shown that both *HA-srs2-R1* (not SUMOylated and unable to bind PCNA) and *srs2-3KR* (not SUMOylated but able to bind PCNA) reverse the rescue of MMS and HU sensitivity conferred by *ULS1* deletion in the *sgs1Δ* mutant. This suggests that not only decreased PCNA binding but also the activity of SUMOylated *Srs2* contribute to the suppression of the phenotypes of *sgs1Δ* and possibly also of *mus81Δ sgs1Δ* mutants. These results support a conclusion, also suggested in previous reports (Saponaro *et al.* 2010; Kolesar *et al.* 2016), that SUMOylated *Srs2* is active in PCNA-independent recombination repair pathways, and not only targeted for degradation. We propose that in *uls1Δ* mutants *Srs2* activity is redirected toward these alternative pathways (Figure 8).

Rad5 has also been shown to mediate postreplication repair by replication fork reversal (Minca and Kowalski 2010) and D-loop invasion (Burkovics *et al.* 2014) independently of *Rad51* and perhaps also of PCNA. Interestingly, we demonstrate that the *Rad5*-dependent pathway is required for the

rescue of the *mus81Δ sgs1Δ* mutant's severe growth defect by deletion of *RAD51* or *ULS1*. *Rad5*, together with *Srs2*-SUMO, is also required for the rescue of the *sgs1Δ* mutant's HU and MMS sensitivity by deletion of *ULS1* (Kramarz *et al.* 2014), so we propose that *Rad5* could participate in the alternative pathway activated in the *uls1Δ* mutant when SUMOylated *Srs2* accumulates.

There is increasing evidence that during unperturbed replication and at very low levels of DNA damage, *Rad18*-dependent DDT is the pathway of choice, with the error-free *Rad5*- (and *Rad51*-) dependent template switch operating during S phase, and error-prone synthesis active mainly on ssDNA gaps in G₂ (Hishida *et al.* 2010; Huang *et al.* 2013; Lehner and Jinks-Robertson 2014). We postulate that this error-free pathway is stimulated by *Uls1*. It is known, however, that SUMO conjugation of PCNA is not triggered exclusively in S phase, but also during severe DNA damage (Hoegge *et al.* 2002), so under such conditions a switch to another pathway of DDT most probably occurs. One candidate pathway has recently been proposed (Urulangodi *et al.* 2015) where *Elg1*, *Esc2*, and the *Slx5*-*Slx8* complex collaborate to limit the *Srs2* antirecombinase activity at the sites of replication-associated damage. Interestingly, *Uls1* and *Slx5* have been shown to interact physically with *Elg1*, but the phenotypes of *elg1Δ uls1Δ* and *elg1Δ slx5Δ* double mutants are different and the *slx5Δ* mutant is sensitive to MMS, while *uls1Δ* is not (Cal-Bąkowska *et al.* 2011; Parnas *et al.* 2011). Interaction with *Slx5* has been proposed to be important for *Uls1* activity, but the relationship between the two proteins was antagonistic (Tan *et al.* 2013). This suggests that *Uls1* and *Slx5* are not redundant, and have unique functions in the cell. We could speculate that the former may operate during low and the latter at high levels of replication stress. This would be consistent with the lack of DNA-damage sensitivity in *uls1Δ* as opposed to the high sensitivity of *slx5Δ*, and the synthetic sickness observed in a double mutant devoid of both *Uls1* and *Slx5* (Kramarz *et al.* 2014).

The evidence is mounting that *Uls1* may play an important role in DNA metabolism by targeting different SUMO-modified proteins. It would thus be very interesting in the future to determine the identity of other *Uls1* partners and elucidate the nature of the interplay between *Uls1*- and *Slx5*-regulated pathways.

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Literature Cited

- Abed, M., K. C. Barry, D. Kenyagin, B. Koltun, T. M. Phippen *et al.*, 2011 Degringolade, a SUMO-targeted ubiquitin ligase, inhibits Hairy/Groucho-mediated repression. *EMBO J.* 30: 1289–1301.
- Armstrong, A. A., F. Mohideen, and C. D. Lima, 2012 Recognition of SUMO-modified PCNA requires tandem receptor motifs in Srs2. *Nature* 483: 59–63.
- Bernstein, K. A., E. Shor, I. Sunjevaric, M. Fumasoni, R. C. Burgess *et al.*, 2009 Sgs1 function in the repair of DNA replication intermediates is separable from its role in homologous recombination repair. *EMBO J.* 28: 915–925.
- Bienko, M., C. M. Green, N. Crosetto, F. Rudolf, G. Zapart *et al.*, 2005 Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. *Science* 310: 1821–1824.
- Blanco, M. G., J. Matos, and S. C. West, 2014 Dual control of Yen1 nuclease activity and cellular localization by Cdk and Cdc14 prevents genome instability. *Mol. Cell* 54: 94–106.
- Branzei, D., F. Vanoli, and M. Foiani, 2008 SUMOylation regulates Rad18-mediated template switch. *Nature* 456: 915–920.
- Burkovics, P., M. Sebesta, A. Sisakova, N. Plaut, V. Szukacsov *et al.*, 2013 Srs2 mediates PCNA-SUMO-dependent inhibition of DNA repair synthesis. *EMBO J.* 32: 742–755.
- Burkovics, P., M. Sebesta, D. Balogh, L. Haracska, and L. Krejci, 2014 Strand invasion by HLTf as a mechanism for template switch in fork rescue. *Nucleic Acids Res.* 42: 1711–1720.
- Cal-Bąkowska, M., I. Litwin, T. Bocér, R. Wysocki, and D. Dziadkowiec, 2011 The Swi2-Snf2-like protein Uls1 is involved in replication stress response. *Nucleic Acids Res.* 39: 8765–8777.
- Cejka, P., J. L. Plank, C. Z. Bachrati, I. D. Hickson, and S. C. Kowalczykowski, 2010 Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. *Nat. Struct. Mol. Biol.* 17: 1377–1382.
- Chi, P., Y. Kwon, M.-L. Visnapuu, I. Lam, S. R. Santa Maria *et al.*, 2011 Analyses of the yeast Rad51 recombinase A265V mutant reveal different in vivo roles of Swi2-like factors. *Nucleic Acids Res.* 39: 6511–6522.
- Collins, S. R., K. M. Miller, N. L. Maas, A. Roguev, J. Fillingham *et al.*, 2007 Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature* 446: 806–810.
- Davies, A. A., D. Huttner, Y. Daigaku, S. Chen, and H. D. Ulrich, 2008 Activation of ubiquitin-dependent DNA damage bypass is mediated by replication protein A. *Mol. Cell* 29: 625–636.
- Fabre, F., A. Chan, W.-D. Heyer, and S. Gangloff, 2002 Alternate pathways involving Sgs1/Top3, Mus81/Mms4, and Srs2 prevent formation of toxic recombination intermediates from single-stranded gaps created by DNA replication. *Proc. Natl. Acad. Sci. USA* 99: 16887–16892.
- Friedl, A. A., B. Liefshitz, R. Steinlauf, and M. Kupiec, 2001 Deletion of the SRS2 gene suppresses elevated recombination and DNA damage sensitivity in *rad5* and *rad18* mutants of *Saccharomyces cerevisiae*. *Mutat. Res. DNA Repair* 486: 137–146.
- Gangloff, S., J. P. McDonald, C. Bendixen, L. Arthur, and R. Rothstein, 1994 The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol. Cell. Biol.* 14: 8391–8398.
- Hishida, T., Y. Hirade, N. Haruta, Y. Kubota, and H. Iwasaki, 2010 Srs2 plays a critical role in reversible G2 arrest upon chronic and low doses of UV irradiation via two distinct homologous recombination-dependent mechanisms in postreplication repair-deficient cells. *Mol. Cell. Biol.* 30: 4840–4850.
- Hoege, C., B. Pfander, G.-L. Moldovan, G. Pyrowolakis, and S. Jentsch, 2002 RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 419: 135–141.
- Hofmann, R. M., and C. M. Pickart, 1999 Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. *Cell* 96: 645–653.
- Huang, D., B. D. Piening, and A. G. Paulovich, 2013 The preference for error-free or error-prone postreplication repair in *Saccharomyces cerevisiae* exposed to low-dose methyl methanesulfonate is cell cycle dependent. *Mol. Cell. Biol.* 33: 1515–1527.
- Ira, G., A. Malkova, G. Liberi, M. Foiani, and J. E. Haber, 2003 Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell* 115: 401–411.
- Klein, H. L., 2001 Mutations in recombinational repair and in checkpoint control genes suppress the lethal combination of *srs2Delta* with other DNA repair genes in *Saccharomyces cerevisiae*. *Genetics* 157: 557–565.
- Kolesar, P., P. Sarangi, V. Altmannova, X. Zhao, and L. Krejci, 2012 Dual roles of the SUMO-interacting motif in the regulation of Srs2 sumoylation. *Nucleic Acids Res.* 40: 7831–7843.
- Kolesar, P., V. Altmannova, S. Silva, M. Lisby, and L. Krejci, 2016 Pro-recombination role of Srs2 protein requires SUMO (small ubiquitin-like modifier) but is independent of PCNA (proliferating cell nuclear antigen) interaction. *J. Biol. Chem.* 291: 7594–7607.
- Kramarz, K., I. Litwin, M. Cal-Bąkowska, B. Szakal, D. Branzei *et al.*, 2014 Swi2/Snf2-like protein Uls1 functions in the Sgs1-dependent pathway of maintenance of rDNA stability and alleviation of replication stress. *DNA Repair (Amst.)* 21: 24–35.
- Krejci L., Komen S. Van, Li Y., Villemain J., Reddy M. S., Klein H. L., Ellenberger T., Sung P., 2003 DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* 423: 305–309.
- Le Breton, C., P. Dupaigne, T. Robert, E. Le Cam, S. Gangloff *et al.*, 2008 Srs2 removes deadly recombination intermediates independently of its interaction with SUMO-modified PCNA. *Nucleic Acids Res.* 36: 4964–4974.
- Lehner, K., and S. Jinks-Robertson, 2014 Shared genetic pathways contribute to the tolerance of endogenous and low-dose exogenous DNA damage in yeast. *Genetics* 198: 519–530.
- León Ortiz, A. M., R. J. D. Reid, J. C. Dittmar, R. Rothstein, A. Nicolas *et al.*, 2011 Srs2 overexpression reveals a helicase-independent role at replication forks that requires diverse cell functions. *DNA Repair (Amst.)* 10: 506–517.
- Lescasse, R., S. Pobiega, I. Callebaut, and S. Marcand, 2013 End-joining inhibition at telomeres requires the translocase and polySUMO-dependent ubiquitin ligase Uls1. *EMBO J.* 32: 805–815.
- Lo, Y.-C., K. S. Paffett, O. Amit, J. A. Clikeman, R. Sterk *et al.*, 2006 Sgs1 regulates gene conversion tract lengths and crossovers independently of its helicase activity. *Mol. Cell. Biol.* 26: 4086–4094.
- Mankouri, H. W., T. J. Craig, and A. Morgan, 2002 *SGS1* is a multicopy suppressor of *srs2*: functional overlap between DNA helicases. *Nucleic Acids Res.* 30: 1103–1113.
- Matos, J., M. G. Blanco, and S. C. West, 2013 Cell-cycle kinases coordinate the resolution of recombination intermediates with chromosome segregation. *Cell Rep.* 4: 76–86.
- Minca, E. C., and D. Kowalski, 2010 Multiple Rad5 activities mediate sister chromatid recombination to bypass DNA damage at stalled replication forks. *Mol. Cell* 38: 649–661.
- Minca, E. C., and D. Kowalski, 2011 Replication fork stalling by bulky DNA damage: localization at active origins and checkpoint modulation. *Nucleic Acids Res.* 39: 2610–2623.
- Mitchel, K., K. Lehner, and S. Jinks-Robertson, 2013 Heteroduplex DNA position defines the roles of the Sgs1, Srs2, and Mph1 helicases in promoting distinct recombination outcomes. *PLoS Genet.* 9: e1003340.
- Miura, T., T. Shibata, and K. Kusano, 2013 Putative antirecombinase Srs2 DNA helicase promotes noncrossover homologous recombination avoiding loss of heterozygosity. *Proc. Natl. Acad. Sci. USA* 110: 16067–16072.

- Moldovan, G.-L., B. Pfander, and S. Jentsch, 2007 PCNA, the maestro of the replication fork. *Cell* 129: 665–679.
- Mullen, J. R., V. Kaliraman, S. S. Ibrahim, and S. J. Brill, 2001 Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. *Genetics* 157: 103–118.
- Mullen, J. R., F. S. Nallaseth, Y. Q. Lan, C. E. Slagle, and S. J. Brill, 2005 Yeast Rmi1/Nce4 controls genome stability as a subunit of the Sgs1-Top3 complex. *Mol. Cell. Biol.* 25: 4476–4487.
- Niu, H., and H. L. Klein, 2016 Multifunctional roles of *Saccharomyces cerevisiae* Srs2 protein in replication, recombination and repair. *FEMS Yeast Res.* DOI: 10.1093/femsyr/fow111.
- Papouli, E., S. H. Chen, A. A. Davies, D. Huttner, L. Krejci *et al.*, 2005 Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol. Cell* 19: 123–133.
- Parker, J. L., and H. D. Ulrich, 2012 A SUMO-interacting motif activates budding yeast ubiquitin ligase Rad18 towards SUMO-modified PCNA. *Nucleic Acids Res.* 40: 11380–11388.
- Parker, J. L., A. Bucci, A. A. Davies, K. Heidrich, H. Windecker *et al.*, 2008 SUMO modification of PCNA is controlled by DNA. *EMBO J.* 27: 2422–2431.
- Parnas, O., R. Amishay, B. Liefshitz, A. Zipin-Roitman, and M. Kupiec, 2011 Elg1, the major subunit of an alternative RFC complex, interacts with SUMO-processing proteins. *Cell Cycle* 10: 2894–2903.
- Pfander, B., G.-L. Moldovan, M. Sacher, C. Hoege, and S. Jentsch, 2005 SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature* 436: 428–433.
- Sale, J. E., 2012 Competition, collaboration and coordination - determining how cells bypass DNA damage. *J. Cell Sci.* 125: 1633–1643.
- Saponaro, M., D. Callahan, X. Zheng, L. Krejci, J. E. Haber *et al.*, 2010 Cdk1 targets Srs2 to complete synthesis-dependent strand annealing and to promote recombinational repair. *PLoS Genet.* 6: e1000858.
- Symington, L. S., 1998 Homologous recombination is required for the viability of *rad27* mutants. *Nucleic Acids Res.* 26: 5589–5595.
- Symington, L. S., R. Rothstein, and M. Lisby, 2014 Mechanisms and regulation of mitotic recombination in *Saccharomyces cerevisiae*. *Genetics* 198: 795–835.
- Szkal, B., and D. Brnzei, 2013 Premature Cdk1/Cdc5/Mus81 pathway activation induces aberrant replication and deleterious crossover. *EMBO J.* 32: 1155–1167.
- Tan, W., Z. Wang, and G. Prelich, 2013 Physical and genetic interactions between Uls1 and the Slx5-Slx8 SUMO-targeted ubiquitin ligase. *G3 (Bethesda)* 3: 771–780.
- Ulrich, H. D., and S. Jentsch, 2000 Two RING finger proteins mediate cooperation between ubiquitin-conjugating enzymes in DNA repair. *EMBO J.* 19: 3388–3397.
- Unk, I., I. Hajdu, A. Blastyak, and L. Haracska, 2010 Role of yeast Rad5 and its human orthologs, HLTf and SHPRH in DNA damage tolerance. *DNA Repair (Amst.)* 9: 257–267.
- Urulangodi, M., M. Sebesta, D. Menolfi, B. Szkal, J. Sollier *et al.*, 2015 Local regulation of the Srs2 helicase by the SUMO-like domain protein Esc2 promotes recombination at sites of stalled replication. *Genes Dev.* 29: 2067–2080.
- Uzunova, K., K. Götsche, M. Miteva, S. R. Weisshaar, C. Glanemann *et al.*, 2007 Ubiquitin-dependent proteolytic control of SUMO conjugates. *J. Biol. Chem.* 282: 34167–34175.
- Vanoli, F., M. Fumasoni, B. Szkal, L. Maloisel, and D. Brnzei, 2010 Replication and recombination factors contributing to recombination-dependent bypass of DNA lesions by template switch. *PLoS Genet.* 6: e1001205.
- Veaute, X., J. Jeusset, C. Soustelle, S. C. Kowalczykowski, C. E. Le *et al.*, 2003 The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* 423: 309–312.
- Xie, Y., E. M. Rubenstein, T. Matt, and M. Hochstrasser, 2010 SUMO-independent in vivo activity of a SUMO-targeted ubiquitin ligase toward a short-lived transcription factor. *Genes Dev.* 24: 893–903.
- Zeman, M. K., and K. A. Cimprich, 2013 Causes and consequences of replication stress. *Nat. Cell Biol.* 16: 2–9.
- Zhang, Z., K. Shibahara, and B. Stillman, 2000 PCNA connects DNA replication to epigenetic inheritance in yeast. *Nature* 408: 221–225.

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