The Shu complex, which contains Rad51 paralogues, promotes DNA repair through inhibition of the Srs2 anti-recombinase

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ABSTRACT The Shu complex, which contains RAD51 paralogues, is involved in the decision between homologous recombination and error-prone repair. We discovered a link to ribosomal DNA (rDNA) recombination when we found an interaction between one member of the Shu complex, SHU1, and UAF30, a component of the upstream activating factor complex (UAF), which regulates rDNA transcription. In the absence of Uaf30, rDNA copy number increases, and this increase depends on several functional subunits of the Shu complex. Furthermore, in the absence of Uaf30, we find that Shu1 and Srs2, an anti-recombinase DNA helicase with which the Shu complex physically interacts, act in the same pathway regulating rDNA recombination. In addition, Shu1 modulates Srs2 recruitment to both induced and spontaneous foci correlating with a decrease in Rad51 foci, demonstrating that the Shu complex is an important regulator of Srs2 activity. Last, we show that Shu1 regulation of Srs2 to double-strand breaks is not restricted to the rDNA, indicating a more general function for the Shu complex in the regulation of Srs2. We propose that the Shu complex shifts the balance of repair toward Rad51 filament stabilization by inhibiting the disassembly reaction of Srs2.

INTRODUCTION The ability to repair broken DNA is critical for genomic protection from mutations and chromosomal rearrangements. When genes required for DNA repair are disrupted, mutations arise that can lead to cancer and cell death. A group of proteins important for genome maintenance is the Shu complex (Shu1, Shu2, Psy3, and Csm2), four interacting proteins in the same epistasis group (Shor et al., 2005; Martin et al., 2006; Mankouri et al., 2007; Ball et al., 2009). Although the Shu complex has been shown genetically to promote DNA repair through homologous recombination (HR), little is known about its function during this process.

The Shu complex was originally discovered in the budding yeast Saccharomyces cerevisiae during a screen for mutants that suppress slow growth due to the absence of Top3, a type I topoisomerase that functions with an accessory protein Rmi1 and the RecQ-like helicase Sgs1 during DNA repair (Shor et al., 2005). One of the functions of the Sgs1–Top3–Rmi1 complex is to resolve DNA intermediates during HR. Subsequent analysis of shu mutants revealed that they also suppress the slow growth of RMI1 deletion strains (Mankouri et al., 2007). Additionally, shu deletions partially rescue the DNA damage sensitivity (methyl methanesulfonate [MMS] or hydroxyurea [HU]) and can suppress the hyperrecombination phenotype associated with loss of TOP3 or SGS1 (Shor et al., 2005; Ball et al., 2009). Therefore the name Shu comes from their ability to “suppress sgs1 HU sensitivity.”

Members of the Shu complex physically interact by yeast two-hybrid, suggesting that they are stably associated (Ito et al., 2001; Shor et al., 2005). Disruption of the individual genes of the Shu complex causes sensitivity to the DNA alkylating agent MMS but not to...
other DNA damaging agents (Shor et al., 2005; Mankouri et al., 2007; Ball et al., 2009). Deletion of SHU leads to a mutator phenotype that is epistatic to deletion of RAD52, suggesting that the Shu proteins are involved in DNA repair through the HR pathway (Huang et al., 2003; Shor et al., 2005). The mutator phenotype is attributable to the action of the translesion synthesis polymerase Rev3 during the error-prone DNA repair process (Shor et al., 2005). Recently the Shu complex was proposed to recruit HR proteins needed during error-free postrecombination repair (Ball et al., 2009). Further analysis has revealed that one gene, SHU1, is in the same epistasis group for MMS sensitivity as the DNA repair genes RAD51 and RAD54 (Shor et al., 2005; Mankouri et al., 2007). Two components of the Shu complex, Shu1 and Psy3, are RAD51 paralogues; therefore the Shu complex may promote recombination in a manner similar to other Rad51-like proteins. Based upon these results, a model has been proposed in which the Shu complex functions by promoting repair of DNA damage through the HR pathway whose DNA intermediates are ultimately resolved by the action of Sgs1–Top3–Rmi1 (Shor et al., 2005; Mankouri et al., 2007).

A study in Schizosaccharomyces pombe revealed that the S. cerevisiae Shu complex is conserved in fission yeast and humans (Martín et al., 2006). Originally the fission yeast Sws1 protein was found during a screen for yeast two-hybrid interactions with Srs2 (Martín et al., 2006). The fission yeast gene was called Sws1, and it contains a SWIM domain likely important for protein–protein/DNA interactions (Makarova et al., 2002). Sws1 is conserved in humans, and they are homologues of budding yeast Shu2, which also interacts with Srs2. The functional significance of this conserved interaction is unknown (Ito et al., 2001; Martín et al., 2006). Although no Csm2 homologue has yet been identified, two other members of the budding yeast complex have homologues in fission yeast and humans, Shu1 is homologous to the fission yeast protein Rlp1 and human protein XRCC2, and the latter two each contain a Walker A motif required for ATP binding and hydrolysis. Psy3 shares homology with Rd1 from fission yeast and RAD51D in humans. Along with Sws1, all three of these homologous proteins have Walker B motifs required for ATP binding and hydrolysis. Interestingly, in humans, XRCC2 and RAD51D, Rad51 paralogues, form a heterodimer with ATPase activity stimulated by DNA (Braybrooke et al., 2000).

The Srs2 protein is a 3′ to 5′ DNA helicase that destabilizes Rad51 nucleoprotein filaments in vitro (Krejci et al., 2003; Veau et al., 2003). Because Srs2 disrupts Rad51-mediated strand invasion, a key step in HR, Srs2 has been described as an “anti-recombinase” protein. In fact, Srs2 plays a central role in both postreplication and step in HR, Srs2 has been described as an “anti-recombinase” protein (Mankouri et al., 2007). Because Srs2 disrupts Rad51-mediated strand invasion, a key effect that is dependent on the rDNA replication fork block protein, Fob1. This increase in rDNA recombination, for the most part, is suppressed by deleting SHU1, thus identifying a novel role for Shu1 at rDNA. Furthermore the absence of UAF30 expands the rDNA array, which is dependent upon Shu1. We also show that Srs2 and Shu1 work in the same pathway to suppress uaf30Δ-induced rDNA recombination. Importantly, we find that disruption of SHU1 causes an increase in the number of cells with Srs2 foci as well as an increase in Srs2 recruitment to an inducible break in either rDNA or on chromosome V, suggesting that the Shu complex promotes recombination by inhibiting Srs2 not only in the rDNA but at other chromosomal loci as well. Together, our results show that the Shu complex functions in HR through its interaction with Srs2, providing the first evidence for the significance of this evolutionarily conserved association and underlying a novel mechanism for regulating Srs2 activity.

RESULTS

SHU1 overexpression is toxic in uaf30Δ strains
To identify novel genes that genetically interact with SHU1, an SDL screen was conducted using an overexpressing SHU1 plasmid introduced into the yeast deletion library. The SHU1 gene was cloned under a copper-inducible promoter into a 2-μm vector. In the presence of increasing copper concentrations, SHU1 expression was induced and visualized by protein blot using a FLAG-tagged Shu1 (Supplemental Figure 1A). Overexpression of SHU1 or SHU1-FLAG does not cause any growth defects (Supplemental Figure 1B) and complements the MMS sensitivity of a shu1Δ strain (Supplemental Figure 1C), demonstrating that the SHU1 plasmids encode functional alleles. The untagged SHU1 plasmid was introduced separately into the ~4800 viable haploid strains of the yeast deletion library, and nine disruptions failed to grow when SHU1 was overexpressed. One of these disruptions, uaf30Δ, was confirmed for its SDL interactions after direct transformation of the SHU1 plasmid into the deletion strain (Figure 1A and Supplemental Figure 1A). An SDL interaction is seen even without the addition of copper, suggesting that the basal level of Shu1 expression, likely due to small amounts of copper in the medium, is sufficient to cause this phenotype. This observation is consistent with the finding that the uninduced SHU1 plasmid suppresses the MMS sensitivity of a shu1Δ strain (Supplemental Figure 1C).

Uaf30 is a nucleolar protein important for regulating rDNA recombination
Uaf30 was originally copurified as a component of the UAF complex, which promotes transcription of the rDNA by RNA polymerase I and represses RNA polymerase II (Siddiqi et al., 2001; Hontz et al., 2008). Because UAF functions in the nucleolus at the rDNA, we
analyzed cells expressing Uaf30-yellow fluorescent protein (YFP) and Top1–cyan fluorescent protein (CFP), a known protein that resides in the nucleolus (Edwards et al., 2000; Huh et al., 2003), for their localization. Figure 1B shows that Uaf30-YFP colocalizes with Top1–CFP in the nucleolus. These results confirm a previously published genome-wide study in which Uaf30 was localized to the nucleolus (Huh et al., 2003) and contradict another genome-wide report in which it was found to be cytoplasmic (Huang et al., 2003).

Because Uaf30 is nucleolar and the absence of other components of the UAF complex results in rDNA expansion, we examined the potential role of UAF30 in rDNA recombination using a marker loss assay. In this assay, the ADE2 and CAN1 genes were inserted into one of the 100–200 rDNA repeats, and recombination frequencies were calculated by measuring the simultaneous loss of both markers. Figure 1C shows that uaf30Δ cells exhibit increased rDNA recombination frequencies compared with the wild-type (WT) parental strain. Furthermore, this increase is dependent upon FOB1, a gene important for rDNA replication fork stalling that leads to a low frequency of spontaneous DSBs in the array (Figure 1C) (Kobayashi et al., 1998, 2004; Burkhalter and Sogo, 2004).

**Shu1 also localizes to the nucleolus and affects rDNA recombination**

Because Uaf30 localizes to the cell nucleolus and uaf30Δ cells are sensitive to SHU1 overexpression, we asked whether Shu1 is also localized to the nucleolus. Previously, we found that a doubly YFP-tagged Shu1 localizes to the nucleus, but we did not look carefully at its nucleolar localization (Shor et al., 2005). Here we show, upon further analysis, that Shu1 is also found in the nucleolus as it colocalizes with Nop1-CFP, a known nucleolar protein (Figure 2A).

Because the Shu complex was previously found to promote recombination through HR (Shor et al., 2005; Mankouri et al., 2007), we hypothesized that in its absence, recombination would be suppressed at the rDNA. Indeed, we found that shu1Δ largely suppresses the increased rDNA recombination of uaf30Δ cells (Figure 2B). In contrast, in the absence of Shu1 alone, rDNA recombination is neither increased nor decreased (Figure 2B and data not shown). These results suggest that Shu1 functions to promote HR processing of rDNA recombination intermediates created by uaf30Δ. Furthermore, these findings are not specific to disruption of SHU1 because disruption of other SHU genes (i.e., SHU2 and CSM2) also lower the increased rDNA recombination frequency of uaf30Δ cells (Supplemental Figure 2). In contrast, deletion of PSY3 does not significantly lower uaf30Δ-increased rDNA recombination and is highly variable (Supplemental Figure 2). In addition, csm2Δ cells alone increase rDNA recombination relative to WT (Supplemental Figure 2). Indeed, different members of the Shu complex can have distinct functions in various processes because differences between Shu complex members have been reported for gross chromosome rearrangements (Huang et al., 2003) and Rad52 focus formation and gene conversion rates in S. pombe (Martin et al., 2006).

Disruption of SHU1 decreases the rDNA recombination levels observed in uaf30Δ cells. To determine whether SHU1 disruption suppresses rDNA instability in general, we analyzed the effect of deleting SHU1 on the increased rDNA recombination that is observed in top1 mutants (Christman et al., 1988; Gangloff et al., 1996). We found that this increased recombination frequency is not significantly altered in a shu1Δ top1Δ double mutant, supporting the notion that the genetic interaction between the Shu complex and Uaf30 is specific (Supplemental Figure 3).

In the absence of the UAF genes, cells expand their rDNA (Nogi et al., 1991; Keys et al., 1996). Because Uaf30 is a component of the UAF complex, we assumed that uaf30Δ strains would exhibit increased rDNA copy number, and indeed they do (Figure 2C). Because Shu1 largely suppresses the recombination frequency observed in uaf30Δ strains (Figure 2B), we hypothesized that its disruption would also suppress the increased size of the rDNA array observed in uaf30Δ. As predicted, shu1Δ uaf30Δ double mutants exhibit a reduced rDNA copy number compared with uaf30Δ (Figure 2C). However, shu1Δ shows a slight increase in rDNA copy number relative to WT (Figure 2C).
Finally, we asked whether overexpressing Shu1 would further increase rDNA recombination because the Shu complex is genetically involved in promoting recombination. Using the copper-inducible SHU1 plasmid characterized in Supplemental Figure 1, we found that the increased rDNA recombination observed in uaf30Δ cells is exacerbated by SHU1 overexpression (Figure 2D). Given that SHU1 overexpression is toxic in a uaf30Δ background, the recombination frequencies may actually be even higher than those observed if recombination events lead to death or growth arrest of these cells.

**Shu1 functions in the same pathway as Srs2**

Two of the Shu complex proteins, Shu1 and Psy3, are Rad51 paralogues. The Rad51 paralogues, along with Rad52, promote Rad51 filament formation. Importantly, in both budding and fission yeast, another member of the Shu complex, Shu2, physically interacts with the Srs2 helicase, which disrupts Rad51 filaments (Ito et al., 2001; Krejci et al., 2003; Veauve et al., 2003; Martin et al., 2006). Genetic and biochemical studies suggest that Srs2 functions as an anti-recombinase regulating Rad51-mediated strand exchange (Aboussekhra et al., 1999; Krejci et al., 2003; Veauve et al., 2003).

Based upon these observations, we hypothesized that the Shu complex and Srs2 may be involved in the same pathway controlling rDNA hyperrecombination in uaf30Δ. Because Srs2 was previously shown to be enriched in the nucleolus (Torres-Rosell et al., 2007), we examined whether srs2Δ mutant cells, like shu1Δ, can also suppress increased rDNA recombination seen in the absence of UAF30. Disruption of SRS2 alone modestly increases the frequency of rDNA recombination (threelfold over WT), consistent with its hyperrecombination phenotype observed at other loci. When combined with a uaf30Δ, the uaf30Δ srs2Δ double mutant shows similar recombination rates to uaf30Δ shu1Δ, indicating that both srs2Δ and shu1Δ suppress the uaf30Δ defect (Figures 3A and 2B). We also find that the uaf30Δ srs2Δ shu1Δ triple mutant exhibits the same level of suppression, suggesting that Srs2 and Shu1 function in the same pathway in response to uaf30Δ-induced DNA damage at the rDNA. Consistent with this view, we do not observe any synthetic growth defect in srs2Δ uaf30Δ double-mutant strains, unlike that reported in a genome-wide study (Pan et al., 2006).

**Shu1 affects Srs2 and Rad51 focus formation both spontaneously and at site-specific breaks**

In vivo, Srs2 forms foci at sites of DNA replication and recombination, where it removes Rad51 nucleoprotein filaments (Burgess et al., 2009). Because Shu1 and Srs2 genetically interact, it is possible that Shu1 normally promotes recombination by inhibiting the anti-recombinase function of Srs2. To test this hypothesis, we analyzed whether the number of Srs2 foci change in shu1Δ, uaf30Δ, or shu1Δ uaf30Δ cells (Figure 3B). Interestingly, shu1Δ and shu1Δ uaf30Δ strains show an increased number of Srs2 foci (p ≤ 0.005; Figure 3B). In contrast, uaf30Δ strains, which exhibit increased rDNA recombination frequency, have fewer Srs2 foci relative to WT (p ≤ 0.05; Figures 1C and 3B). Because shu1Δ cells increase the fragment as described in Materials and Methods. SD are plotted. (D) WT and uaf30Δ strains were transformed with the empty vector (pWJ1530) or the SHU1 overexpression plasmid (pWJ1530-SHU1). Strains were grown in the presence of 100 μM copper (CuSO₄), and the frequency of rDNA recombinants (CAN₉, ade2) was measured and the SE are plotted. Note that the recombination frequencies in uaf30Δ cells were different relative to (B), likely due to their growth in synthetic minimal medium, which was needed to maintain the plasmid.
number of spontaneous Srs2 foci, which may in turn increase Srs2 anti-recombinase activity, we analyzed whether the ability of Rad51 to form recombination foci is impaired by SHU1 disruption (Figure 3C). We find that the number of spontaneous Rad51 foci in a shu1Δ strain is decreased relative to WT (p ≤ 0.05; Figure 3C). Altogether these results are consistent with the notion that, in absence of Shu1, the activity of Srs2 is increased.

To test directly whether Shu1 regulates Srs2 recruitment to DSB sites, we took advantage of a system where an I-SceI endonuclease cut site was inserted into one rDNA repeat (Torres-Rosell et al., 2007) or outside the rDNA at the URA3 locus on chromosome V (Lisby et al., 2004) (Figure 4). In addition, a tandem array of Tet repressor–binding sites (224xTetO or 336xTetO, respectively) was positioned adjacent to each cut site. The localization of the cut site is revealed by expression of TetI fused to a monomeric red fluorescent protein (mRFP), which binds to TetO. Using this system, we analyzed the localization of fluorescently tagged Srs2 or Rad51 with respect to the DNA cut site after inducing a DSB in both WT and shu1Δ cells. Images of Srs2 are shown with white arrowheads indicating foci. Each experiment was done in triplicate with a total of 400–500 cells analyzed. The graph shows the percentage of cells with foci along with the SE. (C) Cells expressing CFP-Rad51 were analyzed in WT and shu1Δ strains for the percentage of spontaneous nuclear foci. Each experiment was done in triplicate with a total of 150–200 cells analyzed with SE plotted. Note that the strains also contain a WT Rad51–complementing plasmid because CFP-Rad51 is not fully functional.
then Rad51 focus formation would be reduced in a few filaments are formed. If the latter explanation were correct, Rad51 filament formation (acting like a mediator), and in its absence, of the Tet repressor–binding sites (336x integrated at the quantitated in the graph with SE plotted. (B) An I- Sce I plasmid. The results are provided the basis for thinking about the general function of this model in which the Shu complex promotes recombination, both within and outside of the rDNA, by inhibiting Srs2 recruitment to damaged DNA and thus stabilizing Rad51 filaments (Figure 6). For

FIGURE 4: Shu1 inhibits Srs2 recruitment to DNA breaks. (A) An I-SceI cut site was integrated into the rDNA adjacent to a tandem array of Tet repressor–binding sites (224x tetO). Location of the rDNA break is revealed by expression of a Tet fusions to mRFP, Rad52-CFP and YFP-Srs2 were monitored for their recruitment to rDNA breaks in WT and shu1Δ cells expressing a GAL-I-SceI plasmid. The results are quantitated in the graph with SE plotted. (B) An I-SceI cut site was integrated at the URA3 locus on chromosome V adjacent to a tandem array of the Tet repressor–binding sites (336x tetO). Rad52-CFP and YFP-Srs2 were monitored in WT and shu1Δ cells for recruitment to the cut site in strains expressing a GAL-I-SceI plasmid. The results are quantitated in the graph with SE plotted. Rad51 filament formation (acting like a mediator), and in its absence, fewer filaments are formed. If the latter explanation were correct, then Rad51 focus formation would be reduced in a shu1Δ, as we observe in Figure 3C, but would also be reduced a shu1Δ srs2Δ double mutant because the presence or absence of Srs2 should not alter Rad51 filament formation. Indeed, when we disrupt the Rad51 filament mediator, RAD55, fewer Rad51 foci are seen in the absence of SRS2 (Supplemental Figure 4; p ≥ 0.025). Alternatively, if Shu1 inhibits Srs2 directly, then Rad51 focus formation would increase in a shu1Δ srs2Δ double mutant relative to WT, which is precisely what we found. We find that the shu1Δ srs2Δ double mutant exhibits as many Rad51 foci as the srs2Δ strain (Figure 5; p ≥ 0.05). Although we have not completely ruled out that the Shu complex plays some role in Rad51 filament formation, our results strongly suggest that a major role of the Shu complex is to inhibit Srs2.

DISCUSSION

The Shu complex, which consists of four proteins—Shu1, Shu2, Csm2, and Psy3—promotes HR and suppresses error-prone repair (Huang et al., 2003; Shor et al., 2005; Mankouri et al., 2007). To decipher the function of the Shu complex during HR, we began by conducting a novel SDL screen by overexpressing Shu1 in the non-essential deletion library. We found that uaf30Δ cells are sensitive to Shu1 overexpression. Because Uaf30 functions at rDNA and genetically interacts with Shu1, we initially focused our attention on rDNA recombination. The rDNA contains many repeated units, a feature that is conserved throughout evolution (Chindamporn et al., 1993; Rustchenko et al., 1993; Cowen et al., 2000). In fact, maintenance of rDNA repeats is essential for general genomic maintenance, and when copies of the rDNA are lost, cells become sensitive to DNA damage (Ide et al., 2010). Recombination plays a critical role in rDNA repeat homeostasis, requiring both the recombination protein Rad52 and the replication fork–blocking protein Fob1 (Kobayashi et al., 1998, 2004; Park et al., 1999; Burkhalter and Sogo, 2004).

Here we used the sensitized uaf30 background to characterize the cellular function of the Shu complex. In UAF30 disrupted cells, decreased rates of rDNA transcription likely cause selective pressure for the expansion of the number of rDNA repeats (Figure 2C). Because Shu1 normally promotes recombination, in its absence, rDNA recombination is decreased in a uaf30Δ background. We find that the anti-recombinase, Srs2, and Shu1 function in the same epistasis group to regulate rDNA recombination in the absence of UAF30 (Figure 3A), where Shu1 normally prevents Srs2 recruitment to DSB sites (Figure 4). Because Srs2 antagonizes Rad51 filament formation (Krejci et al., 2003; Veaute et al., 2003), it is surprising that rDNA marker loss is repressed by SRS2 disruption (Figure 3A). Perhaps, increased levels of Rad51 in the absence of Srs2 promote sister chromatid repair at the expense of single-strand annealing, which is the repair mechanism that often leads to increased marker loss at rDNA (Gangloff et al., 1996). Alternatively, the lesions caused by Fob1-dependent replication fork stalling observed in a uaf30Δ cell might be repaired differently. In any case, deletion of SRS2 can cause both expansions and contractions of repetitive DNA elements, as observed at trinucleotide repeats (Kerrest et al., 1999, 2003; Veaute et al., 2003), suggesting that loss of Srs2 can cause genetic instability via different mechanisms. Finally, we find that overexpression of the SHU1 gene exacerbates the hyperrecombination observed at rDNA in the absence of UAF30 (Figure 2D), resulting in uncontrolled recombination, likely explaining the synthetic interaction that we uncovered.

The genetic interaction between Uaf30 and the Shu complex provided the basis for thinking about the general function of this complex during recombination. Our results are consistent with a model in which the Shu complex promotes recombination, both within and outside of the rDNA, by inhibiting Srs2 recruitment to damaged DNA and thus stabilizing Rad51 filaments (Figure 6). For
does the cell balance these two competing reactions? We propose that the Shu complex, which contains RAD51 paralogues, is an important regulator of this equilibrium by inhibiting Srs2 activity to help stabilize the Rad51 filaments and promote recombination.

Many lines of evidence support the notion that the Shu complex regulates Srs2: 1) Shu2 interacts with Srs2 as does the fission yeast homologue SWS2 (Ito et al., 2001; Martin et al., 2006). 2) shu1Δ cells have more spontaneous Srs2 foci correlating with fewer Rad51 foci. Similarly, mammalian cells with down-regulated SWS1, the Shu2 homologue, also exhibit fewer RAD51 foci (Martin et al., 2006). 3) Shu1 inhibits Srs2 recruitment to an induced DSB whether it is in the rDNA or on chromosome V (Figure 4).

In our model, we have also included the possibility that disruption of the Shu complex directly destabilizes Rad51 filaments, preventing formation of the substrate on which Srs2 acts (the dashed arrow in Figure 6). This view is consistent with the observation that disruption of the other RAD51 paralogues, Rad55 and Rad57, also exhibit fewer Rad51 foci (Fung et al., 2009). Indeed, we find that when one of these mediators, RAD55, is disrupted, fewer Rad51 foci are seen, even in the absence of Srs2 (Supplemental Figure 4). On the other hand, the Shu complex cannot be a major mediator of Rad51 filament formation because shu1Δ srs2Δ double mutants, unlike rad55Δ, maintain the increase in Rad51 foci seen in srs2Δ single mutants (Figure 5).

How might the Shu complex inhibit Srs2? Because Shu2 and Srs2 physically interact in both budding and fission yeast (Ito et al., 2001; Martin et al., 2006), it is possible that the Shu complex inhibits Srs2 function through this physical interaction. This interaction could in turn prevent Srs2 from disrupting Rad51 filaments. Two of the Shu complex components, Shu1 and Psy3, are RAD51 paralogues (XRCC2 and RAD51D, respectively) (Martin et al., 2006). The paralogues may mimic Rad51 and compete for its binding with Srs2, thus sequestering Srs2 from Rad51 filaments and preventing their disassembly. During DNA repair, cells must balance productive error-free repair with...
the disassembly of nonproductive recombination intermediates (Kanaar et al., 2008). Here we propose a novel mechanism for the Shu complex, whereby it shifts the balance of repair toward Rad51 filament stabilization by inhibiting the disassembly reaction of Srs2.

**MATERIALS AND METHODS**

**Strains, plasmids, and media**

The strains used in this study are listed in Supplemental Table 1. They are isogenic with W303 and were derived from the RAD5* strains W1588-4C and W5909-1B (Thomas and Rothenstein, 1989; Zhao et al., 1998). The SHU1 overexpression plasmid was created by digesting the pWJ1530 vector, containing ampilicin and leucine selectable markers and a copper-inducible promoter, with the HpaI restriction enzyme and subsequent calf intestinal phosphatase treatment. Primers with sequence identity to SHU1 and with sequence identity to the vector were used to amplify the SHU1 gene from genomic DNA by PCR. One set of primers also inserted a FLAG tag 3’ to the SHU1 coding sequence, removing the stop codon. The digested vector along with the PCR products was transformed into yeast, and plasmids recombinants were selected by plating on synthetic complete medium lacking leucine (SC-Leu). Plasmid DNA was extracted from the selected yeast colonies and amplified in Escherichia coli. The correct configuration of the plasmid was confirmed by restriction digestion and DNA sequence analysis. Standard procedures were used for making crosses, tetrad dissection, and yeast transformation (LiOAc method) (Sherman et al., 1986). The media was prepared as described, except twice the amount of leucine was used (Sherman et al., 1986).

**SDL screen**

The pWJ1530-SHU1 plasmid and the empty vector pWJ1530 were each transformed into a strain containing all 16 chromosomes with conditionally stable centromeres that were made by insertion of a GAL promoter and a counterselectable URA3 gene proximal to the centromere (Reid et al., 2008). This strain was mated to the yeast deletion library (Winzeler et al., 1999) on yeast peptone dextrose (YPD) plates in quadruplicate. The chromosomes from the donor strain are counterselected by growing the cells on galactose medium lacking leucine (SC-Leu). Plasmid DNA was extracted from the selected yeast colonies and amplified in Escherichia coli. The correct configuration of the plasmid was confirmed by restriction digestion and DNA sequence analysis. Standard procedures were used for making crosses, tetrad dissection, and yeast transformation (LiOAc method) (Sherman et al., 1986). The media was prepared as described, except twice the amount of leucine was used (Sherman et al., 1986).

**Protein analysis**

The W5909-1B parental strain was transformed with pWJ1530-SHU1 or pWJ1530-SHU1-FLAG. Two ml yeast cultures grown overnight in SC-Leu medium containing 0, 50, 100, or 200 μM CuSO4 to an OD600 250 μl were collected and protein lysates made as previously described (Kushnirov, 2000). A 3-μl extract was electrophoresed on a 10% SDS–PAGE gel, transferred to membrane, and protein blotted using anti-FLAG antibodies (1:20,000; Sigma F3165, St. Louis, MO) to detect FLAG tag or anti-Adh1 antibodies (1:20,000; Chemicon AB 1202, Temecula, CA) to detect Adh1.

**Microscopy**

Cells were grown in 5 ml cultures of SC medium plus 100 mg/l adriamycin at 23°C overnight and harvested for microscopy as previously described (Lisby et al., 2001), except that images were captured under a 100x magnification oil immersion objective (1.46 numerical aperture) on a Leica DM5500B upright microscope (Leica Microsystems, Buffalo Grover, NY), illuminated with a 100-W mercury arc lamp and high-efficiency YFP and CFP filter cubes. The images were captured with a Hamamatsu Orca AG cooled digital CCD camera, operated by Volocity software (Improvement, Waltham, MA). Stacks of 11 0.3-μm sections were captured using the following channels and exposure times: differential interference contrast (15 ms), Shu1-YFP-YFP (500 ms), Nop1-CFP (10 ms), Uaf30-YFP (1500 ms), Top1-CFP (250 ms), Fob1-YFP (800 ms), Rad52-CFP (800 ms), CFP-Rad51 (800 ms), YFP-Srs2 (2000 ms), and RFP-rDNA (400 ms). Images were processed and enhanced identically using Volocity software and analyzed for localization and fluorescence intensity with the exception of the shu1Δ strain (Figure 4A), for which the rDNA DSB-RFP contrast was increased to be more readily visible.

**I-SceI induction**

Cells harboring GAL-I-SceI–expressing plasmid (pWJ18111) were grown to early log phase in SC medium lacking tryptophan and containing 2% dextrose. The cells were pelleted, washed with water, and resuspended in synthetic medium lacking tryptophan and uracil containing 2% galactose and 2% raffinose for 2 h at room temperature.

**Recombination assays**

The rDNA recombination assay was performed by analyzing yeast for loss of the ADE2/CAN1 markers inserted into one of the rDNA repeated sequences, as described previously (Fritze et al., 1997). A WT strain harboring this assay (W4314-2C) was crossed to the mutant strains. Segregants that contained both the DNA assay and the deletion of the gene of interest were analyzed. Three separate isolates of each strain were resuspended into YPD medium from SC medium lacking adenine and grown for equal cell doublings to stationary phase (1–2 d). The cultures were diluted into water and plated onto SC medium for total cell counts and onto Can-Arg to select for CAN1 marker loss. After 2–4 d growth (to achieve equal colony sizes), colonies were counted and Can-Arg plates were replica plated onto synthetic medium lacking Ade to determine the frequency of loss of both CAN1 and ADE2 genes. Each recombination frequency was normalized to WT, which was set to one.

**Densitometry of linearized rDNA**

Ten ml WT, uaf30Δ, shu1Δ, and uaf30Δ shu1Δ strains were grown to achieve equal cell doublings (i.e., the same number of generations) to saturation in rich medium (YPD). DNA purified from the yeast strains were treated for 2 h with RNase and the restriction enzyme KpnI, a unique restriction site in each rDNA repeat, and electrophoresed on a 1% agarose gel. The digital photo of the gel was split into lanes, and the ethidium signal was quantitated using the ImageJ program from the National Institutes of Health (Bethesda, MD). The peak from the 9.1-kb rDNA band was normalized to the genomic DNA compression band that runs near the 23-kb marker. Using this assay, both genomic DNA and extrachromosomal circles would be linearized. Each sample was analyzed in triplicate and averaged from three independent experiments.

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