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.**

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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

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Abbreviations used: CFP, cyan fluorescent protein; DSB, double-strand break; HR, homologous recombination; HU, hydroxyurea; MMS, methyl methanesulfonate; mRFP, monomeric red fluorescent protein; PCNA, proliferating cell nuclear antigen; rDNA, ribosomal DNA; SC-Leu, synthetic complete medium lacking leucine; SDL, synthetic dosage lethality; UAF, upstream activating factor; WT, wild-type; YFP, yellow fluorescent protein; YPD, yeast peptone dextrose.

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 postreplication 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 (Martin *et al.*, 2006). Originally the fission yeast Sws1 protein was found during a screen for yeast two-hybrid interactions with Srs2 (Martin *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; Martin *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 Rdl1 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; Veaute *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 double-strand break (DSB) repair (Pfander *et al.*, 2005; Dupaigne *et al.*, 2008; Le Breton *et al.*, 2008). In vivo, Srs2 forms nuclear foci that colocalize with the DNA replication protein proliferating cell nuclear antigen (PCNA) or the DNA repair protein Rad52 (Burgess *et al.*, 2009). The role of Srs2 is best understood in postreplication repair, where it interacts with sumoylated PCNA, which in turn recruits Srs2 to damaged replication templates to prevent recombination (Pfander *et al.*, 2005; Le Breton *et al.*, 2008). How Srs2 is recruited during DSB repair is still unclear.

It is essential to regulate recombination in the ribosomal DNA (rDNA) array to maintain homeostasis of the 100–200 repeated units present in each cell. Misregulation of rDNA recombination has been implicated in both aging and cancer (Kobayashi, 2008), and recently orderly maintenance of rDNA copy number has been implicated in general genomic stability (Ide *et al.*, 2010). Additionally, the number of rDNA repeats is linked to the overall level of rDNA transcription. The upstream activating factor (UAF) complex—consisting of Rrn5, Rrn9, Rrn10, Uaf30, and histones H3 and H4—promotes transcription of the array by RNA polymerase I as well as the repression of any rDNA transcription by RNA polymerase II (Keys *et al.*, 1996; Siddiqi *et al.*, 2001). Absence of any one of the main UAF proteins (Rrn5, Rrn9, or Rrn10) leads to a slow growth phenotype along with a switch to Pol II transcription of rDNA as well as an expansion of the array (Keys *et al.*, 1996). On the other hand, absence of Uaf30 protein reduces the association of other UAF components with the rDNA and also increases the density of transcription units within the array (Siddiqi *et al.*, 2001; Hontz *et al.*, 2008).

Here we report the results from a genetic screen performed to identify synthetic dosage lethality (SDL) interactions due to *SHU1* overexpression. A synthetic interaction was identified when Shu1 was overexpressed in a *UAF30* disruption. We find that *UAF30* disruption causes a substantial increase in rDNA recombination, an 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 *uaf30*Δ-induced rDNA recombination. Importantly, we find that disruption of *SHU1* causes an increase in the percentage 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 *uaf30*Δ 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 *shu1*Δ 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, *uaf30*Δ, 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 *shu1*Δ 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

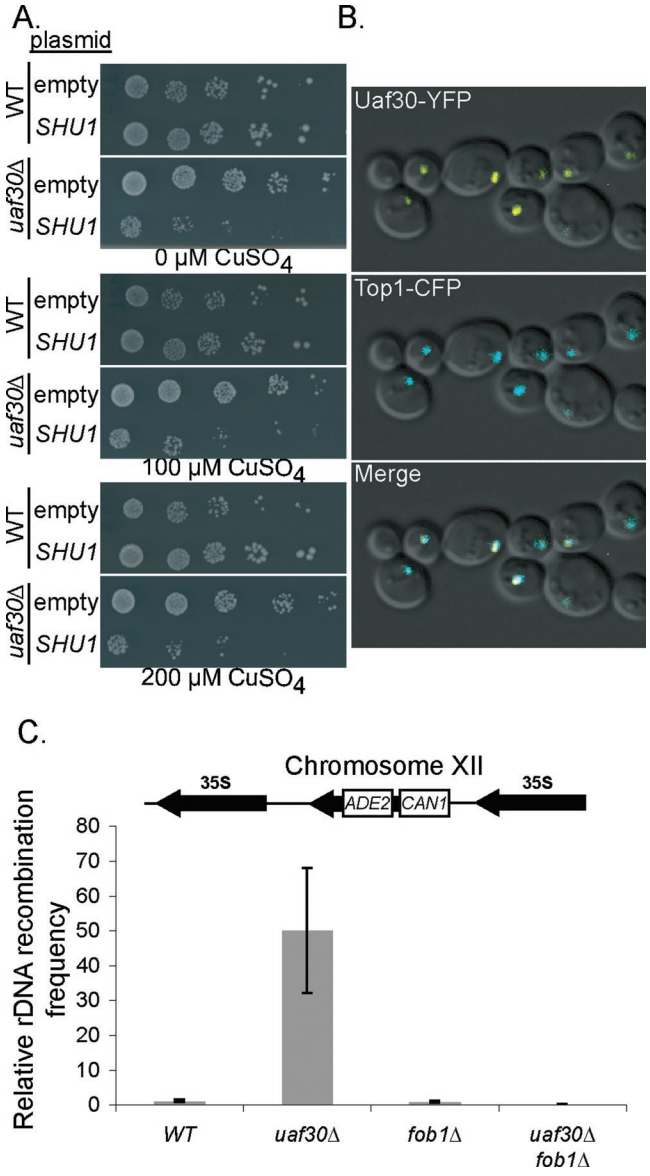

FIGURE 1: Overexpression of *SHU1* causes an SDL interaction with deletion of *UAF30*, a gene that alters rDNA recombination in a *FOB1*-dependent manner. (A) Fivefold serial dilutions were plated onto selective media with increasing copper concentrations with the empty vector (pWJ1530) or the *SHU1* overexpression plasmid (*SHU1*) transformed into WT or *uaf30*Δ. (B) Fluorescence microscopy of yeast cells containing Uaf30-YFP and Top1-CFP was analyzed for colocalization (Merge). (C) The frequency of rDNA recombinants (*CAN*^{R}, *ade2*) was measured in WT, *uaf30*Δ, *fob1*Δ, and *uaf30*Δ *fob1*Δ yeast strains, and they were plotted with SD. Note that rDNA recombination frequencies vary in *uaf30*Δ cells, likely due to the destabilization of the rDNA array.

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 *uaf30*Δ 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 *uaf30*Δ 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 *shu1*Δ largely suppresses the increased rDNA recombination of *uaf30*Δ 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 *uaf30*Δ. 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 *uaf30*Δ cells (Supplemental Figure 2). In contrast, deletion of *PSY3* does not significantly lower *uaf30*Δ-increased rDNA recombination and is highly variable (Supplemental Figure 2). In addition, *csm2*Δ 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 *uaf30*Δ 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 *shu1*Δ *top1*Δ 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 *uaf30*Δ strains would exhibit increased rDNA copy number, and indeed they do (Figure 2C). Because Shu1 largely suppresses the recombination frequency observed in *uaf30*Δ strains (Figure 2B), we hypothesized that its disruption would also suppress the increased size of the rDNA array observed in *uaf30*Δ. As predicted, *shu1*Δ *uaf30*Δ double mutants exhibit a reduced rDNA copy number compared with *uaf30*Δ (Figure 2C). However, *shu1*Δ shows a slight increase in rDNA copy number relative to WT (Figure 2C).

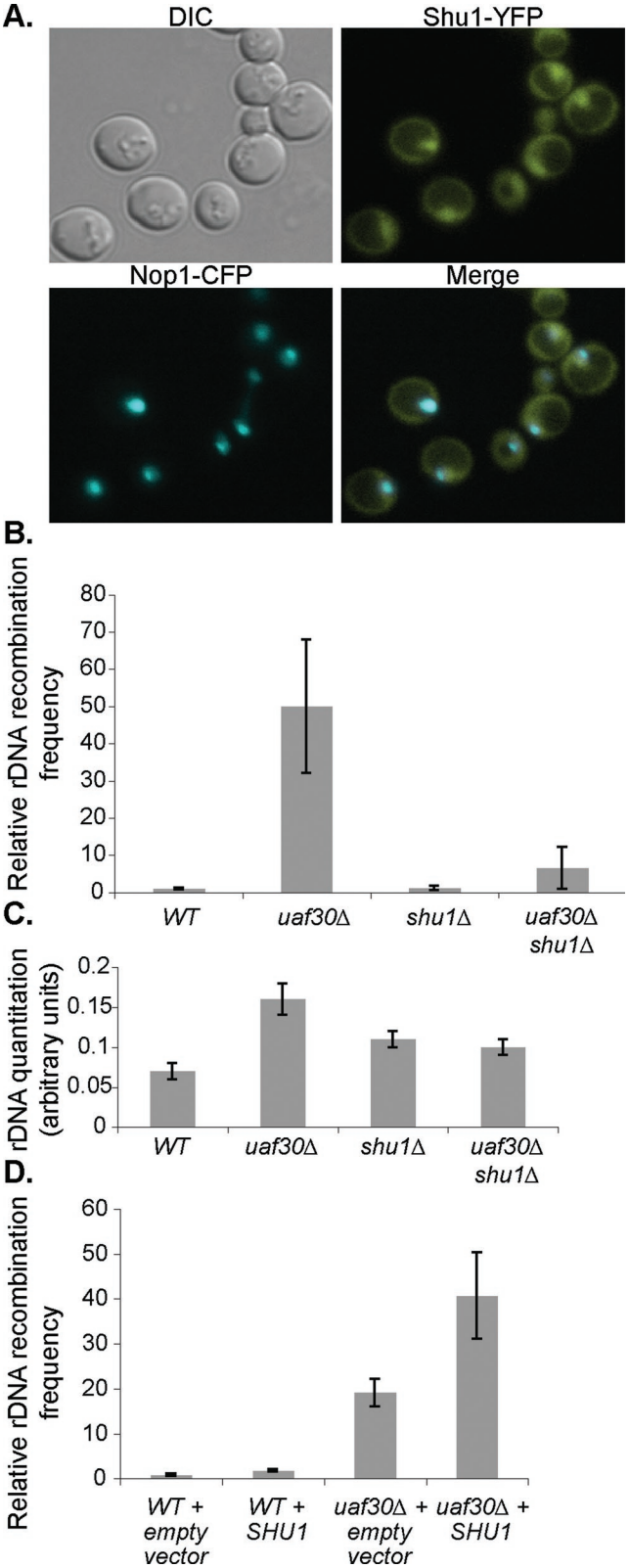

FIGURE 2: Shu1 localizes to the nucleolus and affects rDNA recombination. (A) Yeast with Shu1-YFP-YFP and Nop1-CFP were analyzed by fluorescence microscopy for colocalization (Merge). (B) The frequency of rDNA recombinants (*CAN*^{R}, *ade2*) was measured in WT, *shu1*Δ, *uaf30*Δ, and *uaf30*Δ *shu1*Δ yeast strains, and they were plotted with SD. (C) The amounts of rDNA in WT, *shu1*Δ, *uaf30*Δ, and *uaf30*Δ *shu1*Δ strains were quantitated by analyzing the amount of rDNA resulting from restriction digest of total DNA, revealing a 9.1-kb fragment as described in *Materials and Methods*. SD are plotted. (D) WT and *uaf30*Δ 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 ($CuSO_4$), and the frequency of rDNA recombinants (*CAN*^{R}, *ade2*) was measured and the SE are plotted. Note that the recombination frequencies in *uaf30*Δ cells were different relative to (B), likely due to their growth in synthetic minimal medium, which was needed to maintain the plasmid.

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 *uaf30*Δ cells is exacerbated by *SHU1* overexpression (Figure 2D). Given that *SHU1* overexpression is toxic in a *uaf30*Δ 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; Veaute *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.*, 1989; Krejci *et al.*, 2003; Veaute *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 *uaf30*Δ. Because Srs2 was previously shown to be enriched in the nucleolus (Torres-Rosell *et al.*, 2007), we examined whether *srs2*Δ mutant cells, like *shu1*Δ, can also suppress increased rDNA recombination seen in the absence of *UAF30*. Disruption of *SRS2* alone modestly increases the frequency of rDNA recombination (threefold over WT), consistent with its hyperrecombination phenotype observed at other loci. When combined with a *uaf30*Δ, the *uaf30*Δ *srs2*Δ double mutant shows similar recombination rates to *uaf30*Δ *shu1*Δ, indicating that both *srs2*Δ and *shu1*Δ suppress the *uaf30*Δ defect (Figures 3A and 2B). We also find that the *uaf30*Δ *srs2*Δ *shu1*Δ triple mutant exhibits the same level of suppression, suggesting that Srs2 and Shu1 function in the same pathway in response to *uaf30*Δ-induced DNA damage at the rDNA. Consistent with this view, we do not observe any synthetic growth defect in *srs2*Δ *uaf30*Δ 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 *shu1*Δ, *uaf30*Δ, or *shu1*Δ *uaf30*Δ cells (Figure 3B). Interestingly, *shu1*Δ and *shu1*Δ *uaf30*Δ strains show an increased number of Srs2 foci ($p \leq 0.005$; Figure 3B). In contrast, *uaf30*Δ strains, which exhibit increased rDNA recombination frequency, have fewer Srs2 foci relative to WT ($p \leq 0.05$; Figures 1C and 3B). Because *shu1*Δ cells increase the

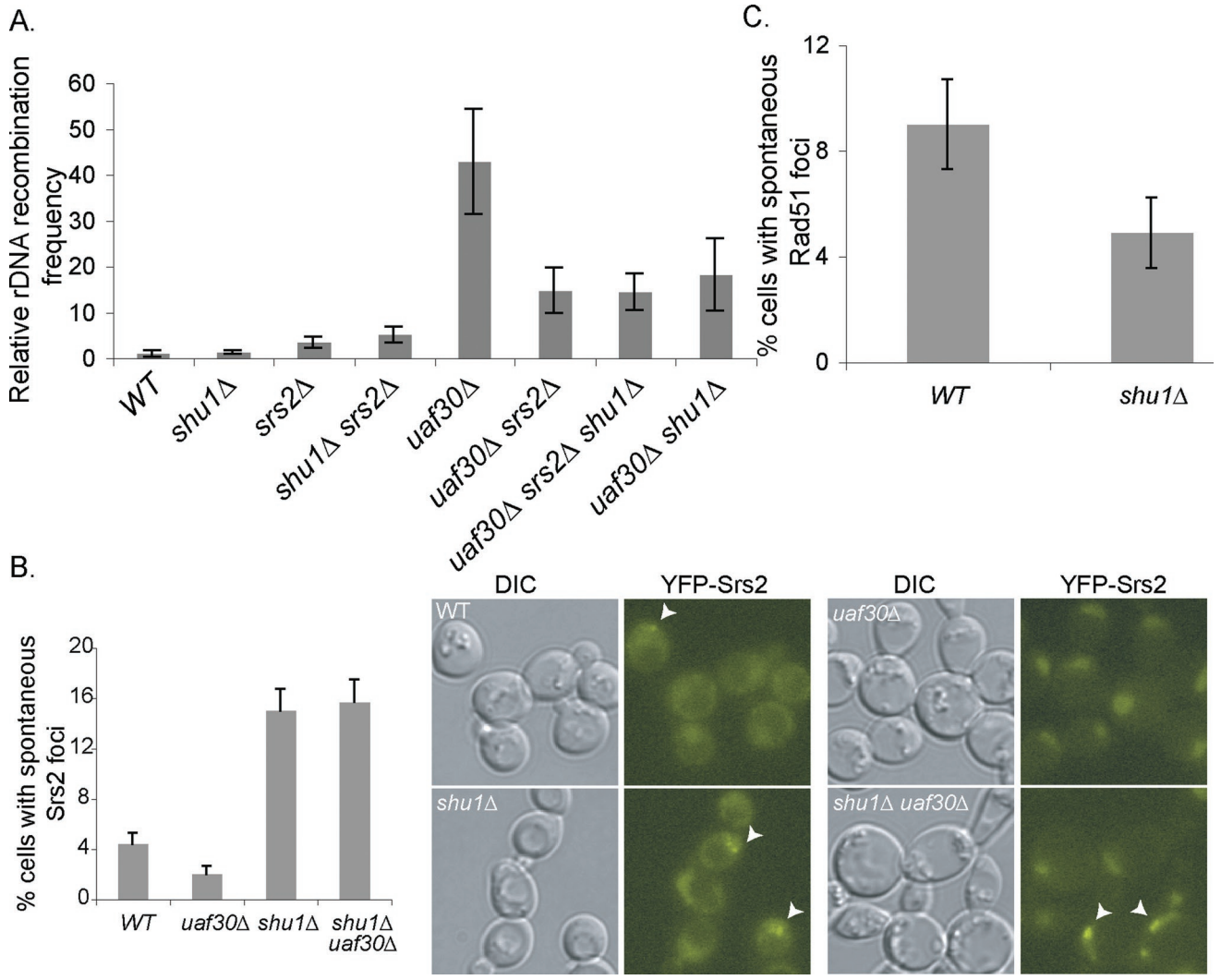

FIGURE 3: Shu1 functions in the same pathway as Srs2 to suppress *uaf30*Δ rDNA recombination and alters Srs2 focus formation. (A) The frequency of rDNA recombination was measured in WT, *shu1*Δ, *srs2*Δ, *shu1*Δ *srs2*Δ, *uaf30*Δ, *uaf30*Δ *srs2*Δ, and *uaf30*Δ *srs2*Δ *shu1*Δ strains, and they were plotted with SD. Note the recombination frequency of the *uaf30*Δ *shu1*Δ strain was not conducted at the same time. (B) YFP-Srs2–expressing strains were analyzed for the percentage of spontaneous nuclear foci in WT, *shu1*Δ, *uaf30*Δ, and *shu1*Δ *uaf30*Δ 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 *shu1*Δ 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.

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 *shu1*Δ strain is decreased relative to WT ($p \leq 0.05$; 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-*Sce*I 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 (224x*tetO* or 336x*tetO*, 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 Rad52 with respect to the DNA cut site after inducing a DSB in both WT and *shu1*Δ cells (Figure 4, A and B). Rad52, a central DNA repair protein, was used to monitor the efficiency of cutting of the DSBs (Lisby *et al.*, 2004; Torres-Rosell *et al.*, 2007). We find that Rad52 is recruited to either the rDNA break or a break in chromosome V, even in the absence of *SHU1* ($p \leq 0.01$). In contrast, Srs2 recruitment to either DSB increases significantly in a *SHU1* disruption (Figure 4, A and B, $p \leq 0.025$ and $p \leq 0.05$, respectively). These results show that Shu1 normally functions to inhibit Srs2 recruitment to DSBs.

The simplest hypothesis to explain these observations is that the Shu complex normally inhibits Srs2, and in its absence, increased Srs2 activity alters the equilibrium to remove Rad51 filaments. An alternative explanation is that the Shu complex is directly involved in

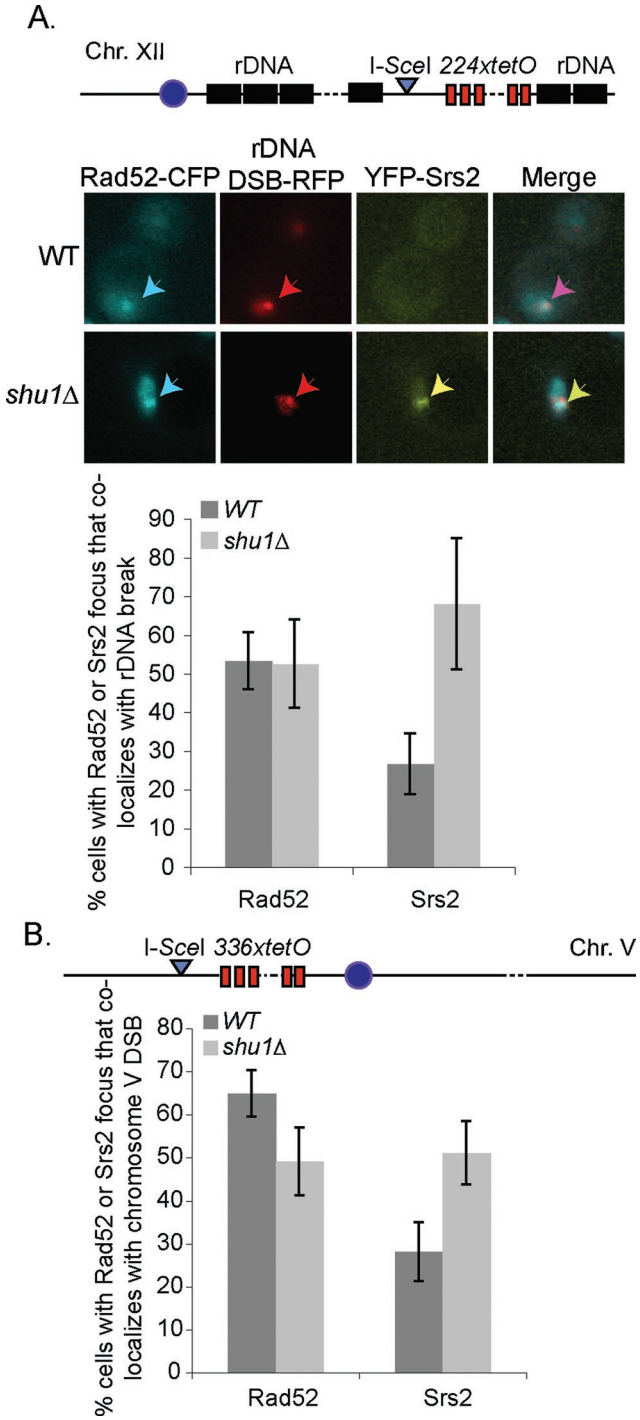

FIGURE 4: Shu1 inhibits Srs2 recruitment to DNA breaks. (A) An I-*Sce*I cut site was integrated into the rDNA adjacent to a tandem array of Tet repressor–binding sites (224x*tetO*). Location of the rDNA break is revealed by expression of a TetI fused to mRFP. Rad52-CFP and YFP-Srs2 were monitored for their recruitment to rDNA breaks in WT and *shu1*Δ cells expressing a GAL-I-*Sce*I plasmid. The results are quantitated in the graph with SE plotted. (B) An I-*Sce*I 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 *shu1*Δ cells for recruitment to the cut site in strains expressing a GAL-I-*Sce*I 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 *shu1*Δ, as we observe in Figure 3C, but would also be reduced a *shu1*Δ *srs2*Δ 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; $p \geq 0.025$). Alternatively, if Shu1 inhibits Srs2 directly, then Rad51 focus formation would increase in a *shu1*Δ *srs2*Δ double mutant relative to WT, which is precisely what we found. We find that the *shu1*Δ *srs2*Δ double mutant exhibits as many Rad51 foci as the *srs2*Δ strain (Figure 5; $p \geq 0.05$). 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 nonessential deletion library. We found that *uaf30*Δ 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 *uaf30*Δ 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 *uaf30*Δ 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.*, 2009), 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

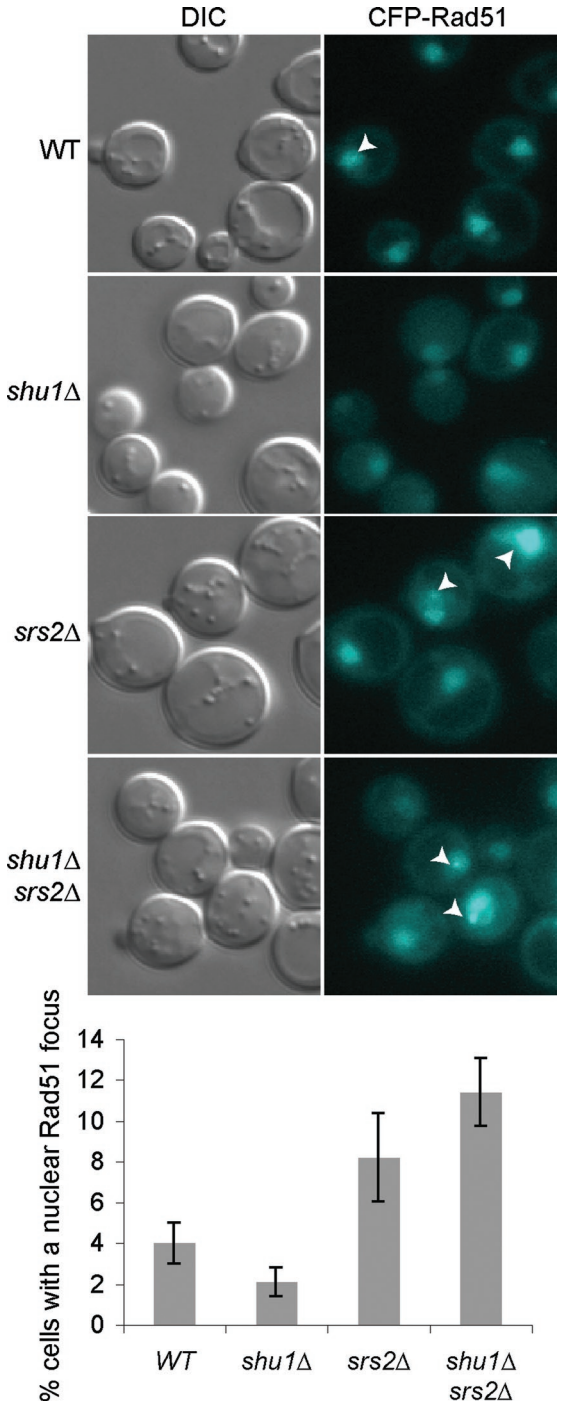

FIGURE 5: Rad51 filament formation is not inhibited in *shu1*Δ *srs2*Δ cells. WT, *shu1*Δ, *srs2*Δ, and *shu1*Δ *srs2*Δ cells were analyzed for the percentage of spontaneous CFP-Rad51 foci. Each experiment was done in triplicate with a total of 200 cells analyzed with SE plotted. Note that the strains also contain a WT Rad51–complementing plasmid because CFP-Rad51 is not fully functional. This configuration likely results in fewer Rad51 foci observed in *srs2*Δ cells than we previously reported (Burgess *et al.*, 2009).

example, in DSB-initiated HR, the DNA ends are resected and processed. The exposed single-stranded DNA is the substrate for Rad51 filament formation. The creation of Rad51 filaments is promoted by Rad52 and its epistasis group of proteins, including the RAD51 paralogues Rad55 and Rad57. At the same time, the filaments can be disassembled by the helicase activity of Srs2. How

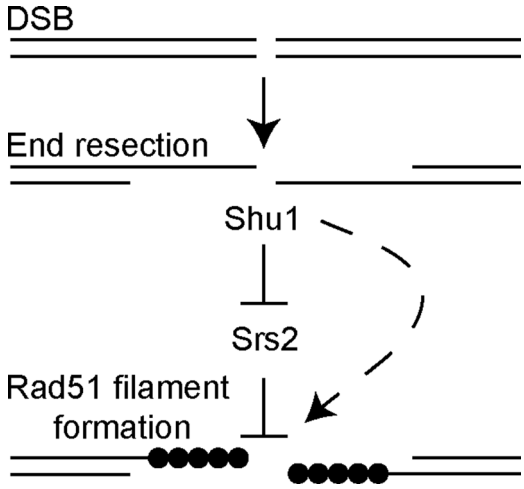

FIGURE 6: Model for repair of DNA breaks regulated by the Shu complex. After a DSB occurs in the DNA, the ends of the break site are resected and processed. Here we show a DSB, but this reaction can take place at any Rad51-mediated step. The Shu complex promotes Rad51 filament formation by inhibiting Srs2 recruitment to the break sites and preventing Srs2 inhibition of Rad51 filament formation. Alternatively, the Shu complex could directly promote Rad51 filament formation in a manner similar to other Rad51 paralogues (dashed line). After Rad51 filaments are formed (indicted by the beads on the single-strand tail), subsequent repair of the DNA lesion can occur via HR.

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) *shu1*Δ 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 *shu1*Δ *srs2*Δ double mutants, unlike *rad55*Δ, maintain the increase in Rad51 foci seen in *srs2*Δ 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 Rothstein, 1989; Zhao *et al.*, 1998). The *SHU1* overexpression plasmid was created by digesting the pWJ1530 vector, containing ampicillin and leucine selectable markers and a copper-inducible promoter, with the *Hpa*I 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 two times and then on SC-Leu medium containing 5FOA with and without 100 μM $CuSO_4$. Yeast deletion strains that grew poorly with *SHU1* overexpression compared with the empty vector were further analyzed. Nine deletion strains were identified and transformed directly with the plasmids, grown overnight in SC-Leu medium, and fivefold serially diluted onto plates with SC-Leu with 0, 50, 100, or 200 μM $CuSO_4$. The plates were photographed after 3 d of growth at 30°C.

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 $CuSO_4$ to an OD_{600}, 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 adenine at 23°C overnight and harvested for microscopy as previously described (Lisby *et al.*, 2001), except that images were captured under a 100× magnification oil immersion objective (1.46 numerical aperture) on a Leica DM5500B upright microscope (Leica Microsystems, Buffalo Grove, 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 (Improvision, 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 *shu1*Δ strain (Figure 4A), for which the rDNA DSB-RFP contrast was increased to be more readily visible.

I-*Sce*I induction

Cells harboring *GAL*-I-*Sce*I–expressing plasmid (pWJ1811) 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 rDNA 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*, *uaf30*Δ, *shu1*Δ, and *uaf30*Δ *shu1*Δ 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 *Kpn*I, 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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