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The Shu complex regulates Rad52 localization during rDNA repair Running Head: Shu1 promotes rDNA repair

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Abstract

The Shu complex, consisting of Rad51 paralogues, is an important regulator of homologous recombination, an error-free DNA repair pathway. Consequently, when members of this complex are disrupted, cells exhibit a mutator phenotype, sensitivity to DNA damage reagents and increased gross chromosomal rearrangements. Previously, we found that the Shu complex plays an important role in ribosomal DNA (rDNA) recombination when the Upstream Activating Factor (UAF) protein Uaf30 is disrupted. *UAF30* encodes a protein needed for rDNA transcription and when deleted, rDNA recombination increases and the rDNA expands in a Shu1-dependent manner. Here we find using the *uaf30*-sensitized background that the central DNA repair protein Rad52, which is normally excluded from the nucleolus, frequently overlaps with the rDNA. This close association of Rad52 with the rDNA is dependent upon Shu1 in a *uaf30* mutant. Previously, it was shown that in the absence of Rad52 sumoylation, Rad52 foci mislocalize to the nucleolus. Interestingly, here we find that using the *uaf30* sensitized background the ability to regulate Rad52 sumoylation is important for Shu1 dependent rDNA recombination as well as Rad52 close association with rDNA. Our results suggest that in the absence of *UAF30*, the Shu complex plays a central role in Rad52 rDNA localization as long as Rad52 can be sumoylated. This discrimination is important for rDNA copy number homeostasis.

Keywords

DNA repair; UAF complex; Shu complex; Rad52; Rad52 sumoylation; rDNA recombination

1. Introduction

Accurate repair of DNA damage is essential to prevent mutations and genomic rearrangements and perturbations of this process are observed in cancer. Repair of repetitive DNA sequences are particularly difficult since their mis-repair can lead to expansions or contractions. One such element is the rDNA, whose organization is a multiple tandem array, a defining feature in most eukaryotic cells [1]. Mis-regulation of rDNA recombination has

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been implicated in both aging and cancer [1]. Maintenance of rDNA copy number is critical for genomic integrity. For example, decreases in rDNA repeats are associated with sensitivity to DNA damaging agents [2]. Therefore, cells have developed mechanisms to repair rDNA breaks to maintain rDNA copy number.

In yeast when the number of rDNA repeats are limiting, the replication fork block located within the rDNA itself can mediate double-strand break (DSB) formation and subsequent recombination for repair [1]. Each rDNA repeat is 9.1 kb long and present in approximately 100-200 copies [3-5]. Unequal sister chromatid exchange between the rDNA repeats can result in expansions as well as contractions of the rDNA [6, 7]. The UAF complex, which contains Rrn5, Rrn9, Rrn10, Uaf30, and histones H3 and H4, is important for promoting rDNA transcription by RNA polymerase I [8, 9]. Absence of UAF complex proteins results in slow growth due to rDNA being transcribed by RNA polymerase II transcription and subsequent increases in repeat number [8]. We previously described how disruption of the UAF complex protein *uaf30* results in increased rDNA recombination that is mediated through the homologous recombination (HR) repair pathway [10]. Interestingly, we find that the hyper-rDNA recombination observed in *uaf30*Δ cells is dependent upon the Shu complex [10], a group of four proteins (Shu1, Shu2, Psy3, and Csm2) that promotes error-free DNA repair through HR [11-15].

Many components of the HR machinery are important for rDNA repair, including the central DNA repair protein Rad52. Surprisingly Rad52 is excluded from the nucleolus, but yet is still crucial for rDNA repair. Therefore a model has been proposed where Rad52-mediated recombination at the rDNA is regulated by moving the rDNA broken ends out of the nucleolus where Rad52 repair centers, observed as foci, can be visualized [16]. In addition, HR at the rDNA is largely dependent upon Rad52 sumoylation and the Smc5-Smc6 complex [16].

Here we report that the absence of *UAF30*, which alters rDNA structure, leads to the close association of Rad52 to the rDNA, a region of the nucleus from which it is normally excluded. We show that in *uaf30* mutant cells, Shu1 promotes Rad52 association with the rDNA. Importantly, Shu1 is critical in regulating Rad52 localization with respect to the rDNA as long as Rad52 can be sumoylated. Furthermore, the ability of Shu1 to regulate rDNA recombination is contingent upon Rad52 localization. Our results suggest a model where the Shu complex promotes rDNA recombination by enabling Rad52 to access the rDNA.

2. Material and methods

2.1. Strains, plasmids, and media

The strains used in this study are listed in Table 1. They are isogenic with W303 and derived from the *RAD5*+ strains W1588-1C and W5909-1B [17, 18]. Standard procedures were used for making crosses, tetrad dissection, and yeast transformation (LiOAc method) [19]. The media was prepared as described, except twice the amount of leucine was added [19].

2.2. Microscopy

Cells were grown overnight in 5 ml cultures of SC medium plus 100 mg/L adenine at 23°C, and harvested for microscopy, as previously described [20]. Images were captured under a 100× magnification oil immersion objective (1.46NA) on a Leica DM5500B upright microscope (Leica Microsystems) illuminated with a 100W mercury arc lamp using 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). Stacks of 11 0.3 micron sections were captured using the following channels and exposure times: DIC (15

ms), Fob1-YFP (800 ms), Fob1-CFP (800 ms), Rad52-CFP (800 ms), and Rad52-KR3-YFP (800 ms). Images were processed and enhanced identically using the Volocity software and analyzed for localization.

2.3. Recombination assays

The rDNA recombination assay was performed by analyzing cells for loss of the *ADE2/CAN1* markers inserted into one of the rDNA repeated sequences, as described [21] see Fig. 3. A wild-type 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 as described [10]. Each recombination frequency was normalized to WT, which was set to one.

3. Results and discussion

3.1. Rad52 localization near rDNA is altered in *uaf30*Δ cells in a Shu1-dependent manner

Rad52 plays a central role in rDNA repair despite its exclusion from the nucleolus [16]. During rDNA repair, the broken ends of the rDNA leave the nucleolus where they have access to the DNA repair machinery, including Rad52 as observed after induction of a single DSB in the rDNA [16]. We asked whether the 30-50 fold increase in rDNA recombination seen in the absence of *UAF30* would use a similar mechanism for repair [10]. Wild-type or *uaf30*Δ cells were examined for the sub-nuclear localization of Rad52-CFP with respect to a protein that marks the rDNA, Fob1-YFP. Consistent with previous reports [16], Fob1 and Rad52 do not co-localize in the parental strain; instead, the two proteins are often visibly separated (Fig. 1A). In contrast, *uaf30*Δ cells often exhibit Fob1 signal that partially overlaps with Rad52 (Fig. 1A, white arrows). This result suggests that when *UAF30* is absent, Rad52 can be more closely associated with the rDNA to aid in the recombinational repair of damage. Perhaps this association is due to the fact that loss of *uaf30* causes the rDNA to be disorganized, which may alter the interface between the nucleolus and the nucleus making it more accessible to Rad52. Consistent with the idea that *uaf30* loss leads to an increased demand for recombination, disruption of *uaf30*Δ leads to synthetic lethality when combined with mutations in many DSB repair genes *(RAD52, MRE11-RAD50-XRS2, TOP3, RMI1, TOP1*; Table 2). Interestingly unlike its synthetic effect with *rad52Δ, uaf30*Δ cells do not exhibit synthetic sickness when combined with *rad51*Δ or *rad54*Δ (Table 2). Perhaps other Rad51-independent pathways, such as single-strand annealing, can also be utilized for rDNA repair in *uaf30* mutants.

Hyper-recombination and rDNA expansion observed in *uaf30*Δ cells are largely suppressed by disrupting *SHU1* [10]. Therefore, we tested whether Rad52 localization with respect to the rDNA would be restored by a *shu1*Δ mutation. In Fig. 1A, *uaf30*Δ and *uaf30Δ shu1*Δ cells were monitored for Fob1-YFP and Rad52-CFP co-localization and compared to the parental strain (WT). Unlike that observed in *uaf30*Δ mutants, Rad52 does not overlap with Fob1 in a *uaf30Δ shu1*Δ double mutant, more closely resembling the association seen in WT strains (Fig. 1A and 1B). These data suggests that *shu1*Δ suppresses *uaf30*Δ rDNA recombination by preventing Rad52-mediated repair in this array.

3.2. Rad52 sumoylation is required for Shu1-dependent Rad52 localization near the rDNA in *uaf30*Δ cells

Sumoylation, an ubiquitin-like modifier, has been previously shown to be important for nuclear organization [22, 23]. Repair of rDNA damage is mediated by non-sumoylated Rad52 [16]. Therefore, we determined whether Rad52 localization, with respect to rDNA, is altered in *uaf30*Δ cells based upon the sumoylation status of Rad52 (Fig. 2A). The *rad52-K43R, K44R, K253R (rad52-KR3)* allele, which has three sumoylation sites of Rad52

mutated, was introduced into our strains [16, 24]. Similar to wild-type Rad52, we find that in *uaf30*Δ cells, the mutant rad52-KR3 protein is closely associated with the rDNA ($p \leq 0.005$; Fig. 2A and 2B). In contrast to what is observed with wild-type Rad52, the rDNA localization of the rad52-KR3 SUMO mutant protein, is largely independent of *SHU1* ($p \leq 0.005$; Fig. 2A and 2B), although there is a small, but significant, restoration of its localization in *uaf30Δ shu1*Δ cells ($p \leq 0.005$). These results suggest that the Shu complex plays a key role in Rad52 rDNA localization when Rad52 can be sumoylated.

3.3. Rad52 sumoylation regulates Shu1-dependent rDNA recombination

We previously showed that *uaf30*Δ cells exhibit increased rDNA recombination frequencies, which are largely dependent upon Shu1 in a Rad52 wild-type background [10]. rDNA recombination was measured in an assay where the *ADE2* and *CAN1* genes were inserted into one of the 100-200 rDNA repeats and recombination frequencies are calculated by determining the simultaneous loss of both markers (Fig. 3A). Consistent with our previous studies, the hyper-recombination observed in *uaf30*Δ cells is largely alleviated by disrupting *shu1*Δ (Fig. 3B; compare *uaf30*Δ to *uaf30Δ shu1*Δ)[10]. When the three lysines of Rad52 in the *rad52-KR3* allele cannot be sumoylated, its localization is comparatively unaffected by absence of *SHU1* (Fig. 2A and 2B). Therefore, we hypothesized that since unsumoylated Rad52 is still closely associated with the rDNA, then the increased rDNA recombination frequency observed in a *uaf30*Δ cell would not be alleviated by *SHU1* disruption. Indeed, we find *uaf30Δ rad52-KR3* double mutant cells maintain increased recombination frequencies, even in the absence of *SHU1* ($p \leq 0.1$; Fig. 3B, compare *uaf30*Δ to *rad52-KR3 uaf30Δ shu1*Δ). Altogether, these results suggest that Shu1 plays a key role in regulating rDNA recombination as well as Rad52 localization as long as Rad52 can be sumoylated.

3.4. Concluding remarks

The Shu complex, consisting of Shu1, Shu2, Csm2, and Psy3, promotes homologous recombination while suppressing error-prone repair [11, 12, 15, 25]. Here we characterize a novel role for Shu1 in the repair of *uaf30*Δ-induced rDNA recombination through its role in the localization of Rad52. When rDNA is destabilized in *uaf30* mutants, we find that Shu1 promotes Rad52/rDNA co-localization. Interestingly, Shu1-dependent regulation of the Rad52 association with rDNA is largely dependent upon Rad52 sumoylation at lysines 43, 44 and 253. The rDNA can be repaired through multiple mechanisms such as sister-chromatid exchange and single-strand annealing, which both utilize Rad52. Since *uaf30*Δ cells exhibit increased rDNA copy number [10], unequal sister chromatid gene conversion is likely the predominant mechanism in mediating the rDNA expansions observed [7].

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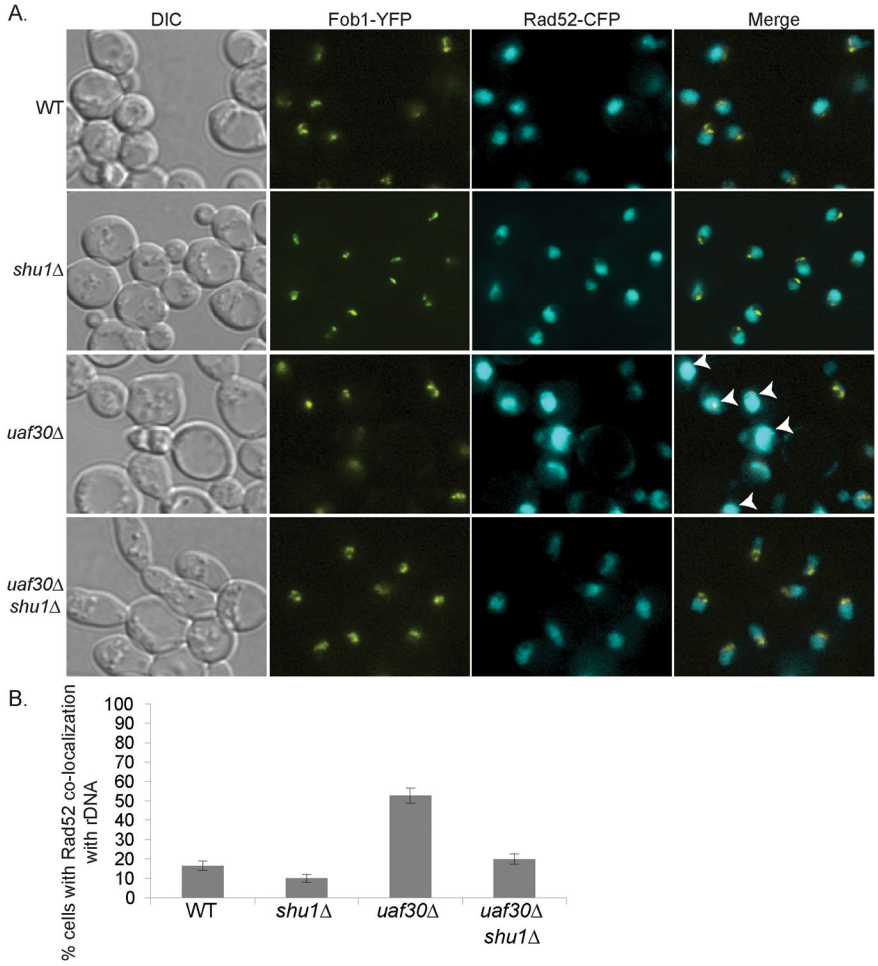

Fig. 1.

Rad52 localization near rDNA is altered in *uaf30*Δ cells in a Shu1-dependent manner. (A) Wild-type (WT), *uaf30*Δ, *uaf30*Δ *shu1*Δ, and *shu1*Δ strains were analyzed for Fob1-YFP and Rad52-CFP co-localization (Merge). In WT and *shu1*Δ cells, there is a separation between the bulk of the Rad52 fluorescence from that of Fob1. In *uaf30*Δ, the demarcation between the two proteins is diminished ($p \leq 0.005$) and is quantitated in (B). Arrows indicate the absence of an interface between Rad52 and Fob1 signals in the cell.

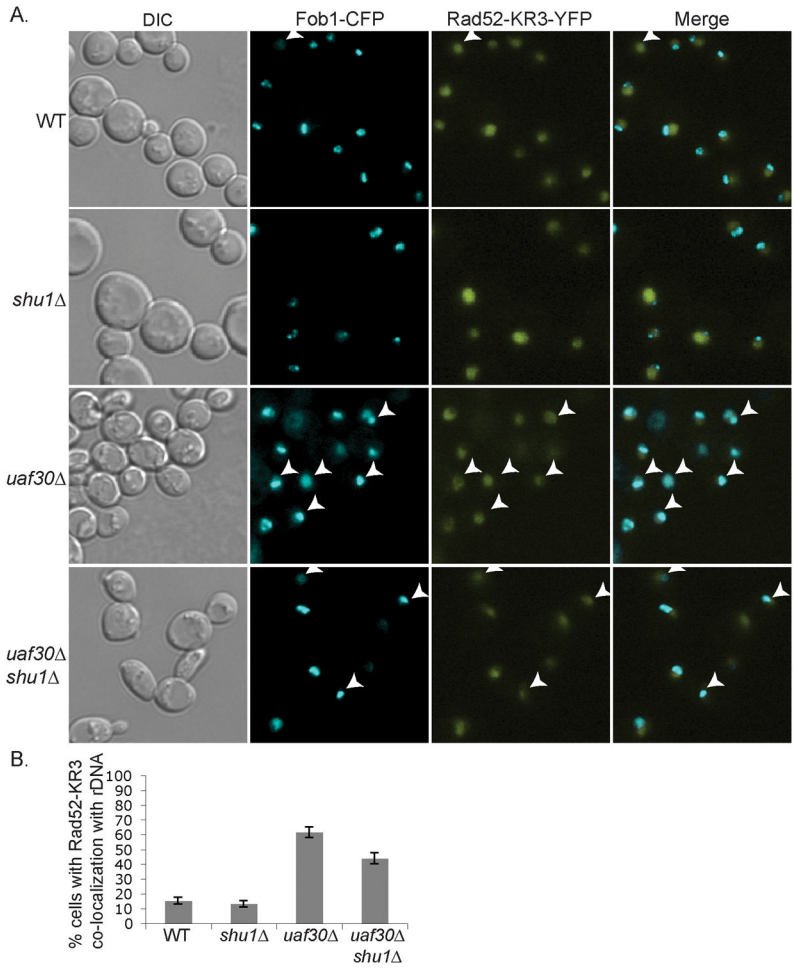

Fig. 2.

Localization of Rad52-KR3 near the rDNA is largely Shu1-independent in *uaf30*Δ cells. (A) Wild-type, *shu1*Δ *uaf30*Δ, and *uaf30*Δ *shu1*Δ strains were analyzed for Fob1-CFP and Rad52-KR3-YFP co-localization (labeled as Merge). (B) Quantitation of the co-localization of Fob1-CFP and Rad52-KR3-YFP with standard errors plotted.

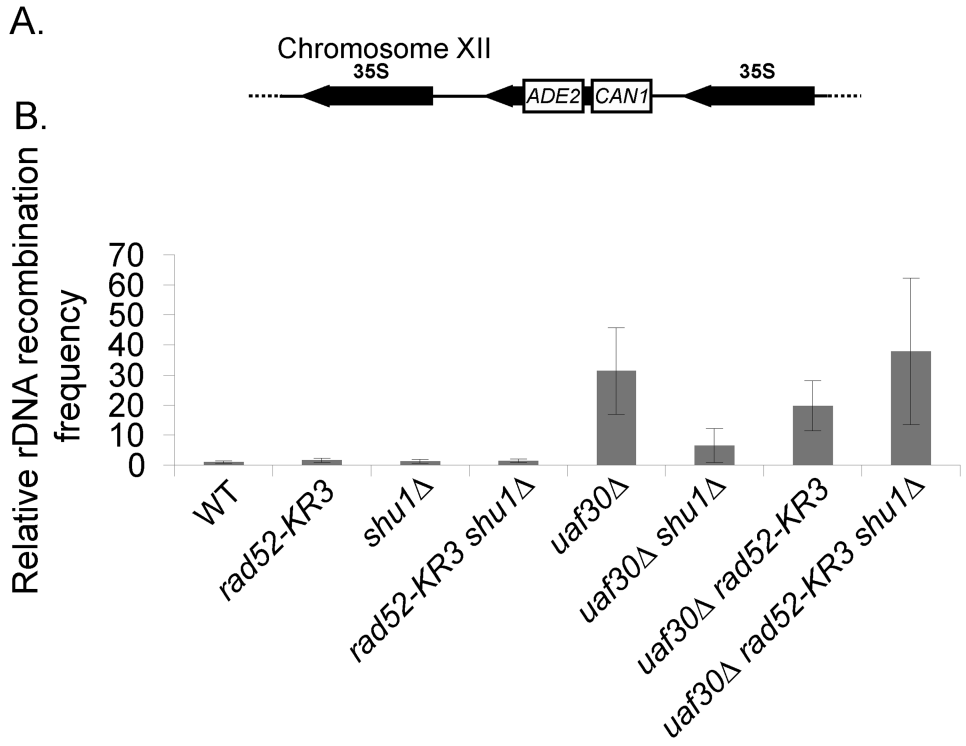

Fig. 3.

Recombination rates remain elevated in a *uaf30Δ shu1*Δ strain in Rad52-KR3. (A) Schematic of rDNA recombination assay used [21]. (B) The frequency of rDNA recombinants (*CAN*^{R}*, ade2*) was measured in WT, *rad52-KR3, shu1*Δ, *rad52-KR3 shu1*Δ, *uaf30*Δ, *uaf30*Δ *shu1*Δ, *uaf30*Δ *rad52-KR3* and *uaf30*Δ *rad52-KR3 shu1*Δ yeast strains with standard deviations plotted.

Table 1

Yeast Strains and Plasmids

Name	Description
W5909-1B	*MAT***a** *TRP1 ADE2 his3-11,15 leu2-3,112 ura3-1 lys2Δ RAD5*
W1588-4A	*MATα leu2-3,112 ade2-1 can1-100 his3-11,15 ura3-1 trp1 LYS2 MET14 RAD5*
W4314-2C	*MATα rDNA(: :)ADE2-CAN1*
W8284-3D	*MATα uaf30(: :)URA3 rDNA(: :)ADE2-CAN1*
W8284-18B	*MATα uaf30(: :)URA3 shu1(: :)HIS3 rDNA(: :)ADE2-CAN1*
W8284-9B	*MATα shu1(: :)HIS3 rDNA(: :)ADE2-CAN1*
W5204-1B	*MAT***a** *FOB1-YFP RAD52-CFP ADE2 bar1(: :)LEU2*
W8442-18A	*MAT***a** *uaf30(: :)URA3 FOB1-YFP RAD52-CFP bar1(: :)LEU2*
W9073-16C	*MAT***a** *uaf30(: :)URA3 shu1(: :)HIS3 FOB1-YFP RAD52-CFP bar1(: :)LEU2*
W9137-14D	*MAT***a** *shu1(: :)HIS3 bar1(: :)LEU2 FOB1-YFP RAD52-CFP*
W9069-4B	*MAT***a** *rad52-K43R,K44R,K253-YFP FOB1-CFP bar1(: :)LEU2*
W9069-4D	*MATα rad52-K43R,K44R,K253R-YFP FOB1-CFP uaf30(: :)URA3 shu1(: :)HIS3*
W9069-9D	*MATα rad52-K43R,K44R,K253R-YFP FOB1-CFP shu1(: :)HIS3*
W9069-22B	*MATα rad52-K43R,K44R,K253R-YFP FOB1-CFP uaf30(: :)URA3*
W9085-17D	*MAT***a** *rDNA(: :)ADE2-CAN1 rad52-K43R,K44R,K253R-YFP*
W9085-8D	*MATα rDNA(: :)ADE2-CAN1 rad52-K43R,K44R,K253R-YFP shu1(: :)HIS3*
W9085-17A	*MATα rDNA(: :)ADE2-CAN1 rad52-K43R,K44R,K253R-YFP uaf30(: :)URA3*
W9085-3D	*MAT***a** *rDNA(: :)ADE2-CAN1 rad52-K43R,K44R,K253R-YFP shu1(: :)HIS3 uaf30(: :)URA3*

All yeast strains are RAD5 derivatives of the W303 background [17] W1588-4A [18] with only the relevant genotype shown.

Table 2
*uaf30*Δ synthetic sick/lethal interacting genes

Mutation	Growth
Recombination:	
*top1*Δ	−
*rmi1*Δ	−
*top3*Δ	−
*mre11*Δ	−
*xrs2*Δ	−
*rad50*Δ	−
*rad52*Δ	−
*shu1*Δ	+
*srs2*Δ	+
*rad51*Δ	+
*rad54*Δ	+
*rdh54*Δ	+
Checkpoint:	
*rad9*Δ	+
*mec1*Δ *sml1*Δ	+
*tof2*Δ	+
*mad2*Δ	+

All strains were combined with a *uaf30*Δ. The (-) sign indicates a lack of cell growth where the (+) sign indicates wild-type growth.