Blocking target of rapamycin signaling by starvation or rapamycin inhibits ribosomal DNA (rDNA) transcription and causes condensin-mediated rDNA condensation and nucleolar contraction. In the absence of condensin, however, repression of rDNA transcription leads to rDNA instability and elevated level of extrachromosomal rDNA circles and nucleolar fragmentation. Here, we show that mutations in the Rad52 homologous recombination machinery block rDNA instability. Rad52 is normally excluded from the nucleolus. In the absence of condensin, however, repression of rDNA transcription results in Rad52 localization to the nucleolus, association with rDNA and subsequent formation of extrachromosomal rDNA circles, and reduced cell survival. In contrast, deletion of RAD52 restores cell viability under the same conditions. These results reveal an opposing role of condensin and Rad52 in the control of rDNA stability under nutrient starvation conditions.

In Saccharomyces cerevisiae, there are ~150 copies of rDNA tandem repeats on chromosome XII. Each rDNA repeat contains a 35 S and 5 S rRNA gene that is transcribed by RNA polymerases I (Pol I) and III (Pol III), respectively. rDNA is also the nucleolar organizer essential for normal nucleolar structure and functions. Ribosome biogenesis, including rRNA synthesis, processing, and ribosomal particle assembly, occurs in the nucleolus. Therefore, rDNA plays a crucial role in growth regulation (1).

Because of its highly repetitive nature, the rDNA locus is inherently unstable due to various forms of homologous recombination within rDNA array. Maintaining rDNA stability is not only important for cell growth but also for the stability of the whole genome (2). The extrachromosomal rDNA circle (ERC) is a hallmark for rDNA instability (3). ERC is formed when the free DNA end after the double-strand break (DSB) invades a complementary sequence within its own rDNA array, a process known as intrachromosomal homologous recombination. Depending on which rDNA repeat is used for repair, ERC can contain various numbers of rDNA repeats after recombination repair, causing a loss of the same number of repeats from the chromosomal rDNA array. Nevertheless, most cells can manage to maintain rDNA stability, as indicated by their relatively constant numbers of rDNA repeats and the lack of detectable ERCs. However, because of certain gene mutations or under pathological situations such as aging, the rDNA repeat number can be fluctuated accompanied by ERC accumulation. For example, mutation in genes such as SIR2, FOB1, TOP1/2, and RPA135 are known to cause ERC accumulation and/or shrinkage of rDNA array (4–7).

Mutations of Pol I essential subunits or the Pol I transcription factor can enhance homologous recombination and lead to rDNA instability. Brewer and colleagues (6) showed that >80% of the chromosomal copies of the rDNA repeats are deleted in a rpa135 mutant. (RPA135 encodes the second largest Pol I subunit.) Another study showed that in an rpa135 mutant, in which 35 S rRNA is synthesized by Pol II from a multicopy plasmid, more than one-half of the chromosomal rDNA repeats were reduced under the condition that represses Pol I transcription (4). We also observed that inactivation of rDNA transcription by a mutation of RRN3 (which encodes an essential Pol I transcription factor) or RPA190 (which encodes the largest Pol I subunit) leads to nucleolar fragmentation and ERC formation. Consistent with these observations, a top1top2 double mutant shows a substantial inhibition of rRNA synthesis and an unstable rDNA phenotype as revealed by an increased level of ERC (5). These results suggest that active rDNA transcription has a role in maintaining rDNA stability.

Target of rapamycin (TOR) is a central component of nutrient signaling that regulates cell growth. TOR is a conserved phosphatidylinositol 3-kinase-related kinase and a key regulator of ribosome biogenesis (9). TOR is present in two distinct complexes called TOR complex 1 and 2 (TORC1 and TORC2) (10). Rapamycin specifically inhibits TORC1 function. Conditions that inactivate TORC1, such as nutrient starvation or rapamycin treatment, inhibit Pol I- and Pol III-dependent rDNA transcription (11, 12). Inhibition of TORC1 also causes nucleolar contraction and rDNA condensation, which is mediated by condensins (3, 13, 14). Condensin is a highly conserved protein machinery known for condensation of chromosomal DNA and segregation of sister chromatids during cell division (15–17). Upon TORC1 inhibition, condensin is rapidly relocated to the nucleolus and loaded to rDNA tandem repeats, resulting in rDNA compaction. A major role of such rDNA
condensation is to maintain rDNA stability as the absence of condensin during starvation leads to elevated ERCs (14). Because rDNA instability and dysregulation of nucleolar functions have been linked to aging, genomic instability, and cancer (2, 18), it is important to understand the molecular mechanisms that cause rDNA instability. The aim of the present study is to identify the factors that are responsible for rDNA instability and ERC formation. Here, we provide evidence that condensin and Rad52 act antagonistically in the control of rDNA stability.

EXPERIMENTAL PROCEDURES

Yeast Strains and Antibodies—The genotypes of yeast strains are shown in Table 1. The double mutants (except rpa190-1 rad52Δ) were constructed as described previously (14). rpa190-1 rad52Δ was generated by one-step PCR-based gene deletion as described previously (19). RAD52-HA6 is constructed on the pRS314 plasmid under the control of the RAD52 native promoter. The antibodies used were mouse anti-Nop1 (EnCor Biotechnology); Alexa Fluor 594-conjugated goat anti-mouse and Alexa Fluor 488-conjugated goat anti-rabbit (Invitrogen); rabbit polyclonal anti-HA (Bethyl Laboratories); monoclonal anti-HA (12CA5; Harlan Laboratories); and rat anti-tubulin (Sigma).

Indirect Immunofluorescence (IF) Microscopy—Yeast IF experiments were performed as described (20). Primary antibody dilution used are as follows: 1:1,000 anti-Nop1, 1:100 monoclonal anti-HA (12CA5), and 1:500 rabbit polyclonal anti-HA. The antibody-antigen complexes were detected with 1:200 Alexa Fluor 594- or Alexa Fluor 488-conjugated secondary antibodies. DNA was stained with 50 ng/ml DAPI in anti-fade mounting medium for 15 min. Fluorescence signals were analyzed using an Olympus fluorescence microscope equipped with a digital camera.

Cell Extracts and Western Blot Analysis—Cells were lysed with glass beads by vortexing in disruption buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, plus a mixture of protease inhibitors; Roche Applied Science). Protein samples (5–10 μg) were separated on an SDS-polyacrylamide gel and then transferred to polyvinylidene difluoride membranes. The primary antibodies used were as follows: 1:1,000 anti-HA (12CA5) and 1:1,000 anti-tubulin.

Chromatin Immunoprecipitation (ChIP) Assay—ChIP assay was performed as described previously (21). For immunoprecipitations, 1 mg of total protein was incubated with 10 μl of anti-hemagglutinin (HA) for overnight at 4 °C. Protein G-Sepharose beads were used to recover the antibody-antigen-DNA complexes in all experiments. The total input DNA (see Fig. 4A, Input) was prepared in the same way, except that the antibody immunoprecipitation steps were omitted. The isogenic strains without tagged protein were used as a control to access the background PCR signals. The primer pairs for PCR detection were described previously (14).

Analysis of Extrachromosomal rDNA Circles (ERC)—Isolation of DNA and Southern blot analysis for detection of ERC was performed as described previously (14).

Cell Survival Assay—Yeast viability was determined by plating assay as described (22) with minor modification. Briefly, yeast cells were counted with a hemocytometer, and 200 cells were spread on 2% agar yeast peptone dextrose (YPD) plates for 5 days at permissive temperature (23 °C). Colonies were counted, and percent colony formation was calculated. Cell viability was compared with the corresponding untreated control. Each experiment was performed with triplicate samples.

RESULTS

Rad52 Is Required for ERC Formation due to Pol I Inactivation—We have previously observed an rDNA instability in Pol I mutants when Pol I transcription is repressed, whereas rDNA is not properly condensed (14). This observation suggests that there is hyper-recombination in this chromosomal region. ERC is the by-product of hyperactive homologous recombination (HR) within the rDNA array (23). In budding yeast, Fob1 and Rad52 are known to be involved in intra- and interchromosomal rDNA HRs. Fob1 is a replication fork barrier binding protein that is required for rDNA DSB and subsequent HR (4). Aging cells accumulate a large number of
Regulation of rDNA Stability

A

\[ \begin{array}{ccc}
23^\circ C & 37^\circ C \\
\hline
Rap & WT & rpa190-1 \\
- & + & - \\
\hline
& WT & rpa190-1 \\
& - & + \\
& - & - \\
& - & + \\
\end{array} \]

B

\[ \begin{array}{cc}
23^\circ C & 37^\circ C \\
\hline
rpa190-1 & rpa190-1 \\
& rad52A \\
rpa190-1 & fob1A \\
fob1A & \\
\end{array} \]

C

\[ \begin{array}{cc}
23^\circ C & 37^\circ C \\
\hline
\text{rpa190-1} & \\
\text{rpa190-1 rad52A} & \\
\text{rpa190-1 fob1A} & \\
\text{fob1A} & \\
\end{array} \]

D

\[ \begin{array}{cc}
23^\circ C & 37^\circ C \\
\hline
\text{WT} & \\
+\text{Vector} & \\
\text{rpa190-1} & \\
+\text{Vector} & \\
\text{rpa190-1 rad52A} & \\
+\text{Vector} & \\
\text{rpa190-1 rad52A} & \\
+\text{RAD52-HA} & \\
\end{array} \]

E

\[ \begin{array}{cc}
23^\circ C & 37^\circ C \\
\hline
\text{WT} & \\
\text{rpa190-1} & \\
\text{rpa190-1 rad52A} & \\
\text{rpa190-1 fob1A} & \\
\text{rpa190-1 rad52A} & \\
\text{RAD52-HA} & \\
\end{array} \]
ERCs, which can be prevented by a fob1Δ mutation (24). Rad52 is a key conserved component of the recombination machinery for most HR events in yeast and humans (25). Rad52 has also been implicated in ERC accumulation with age (26) and spontaneous ERC formation in young yeast cells (27). We have shown previously that inhibition of rDNA transcription in uncondensed rDNA mutants leads to nucleolar fragmentation and elevated ERC levels (3, 14). Such conditions include inactivation of Pol I temperature-sensitive mutants (such as rpa190-1) in a rich nutrient medium in which the nucleolus remains uncondensed or treatment of a condensin mutant (e.g. ycs4-2) with rapamycin (14). Using genomic DNA Southern blotting, we found an elevated ERC formation in rpa190-1 cells at the restrictive temperature, which was suppressed by rapa-

**FIGURE 1.** Rad52, but not Fob1, is required for rDNA instability in the RNA Pol I mutant. A, inactivation of Pol I causes elevated ERC formation, which is suppressed by rapamycin. Early log phase WT (NOY260) and rpa190-1 (NOY259) strains in YPD medium were incubated in 23 °C or shifted to 37 °C in the absence or presence of 200 nm rapamycin (Rap) for 5 h. Total genomic DNA was isolated, separated by agarose DNA gel electrophoresis, and analyzed by Southern blotting with a 32P-labeled 25 S rDNA probe. Top1top2-4 cells were used as a positive control for ERCs. Chrom, chromosomal. B, deletion of RAD52, but not FOB1, blocks the nucleolar fragmentation phenotype in the ycs4-2 condensin mutant. Images show the IF results from the experiment described in A. Nucleolar structure is shown in light gray, and the nucleus is shown in dark gray. C, quantification of cells showing fragmented nucleolus from the results in B (n > 100).

**FIGURE 2.** Rad52 is required for rDNA instability in condensin mutant treated with rapamycin. A, a schematic representation showing the experimental procedure is shown. Early log cells of ycs4-2 (ZW206), ycs4-2 rad52Δ (SZy1822), ycs4-2 fob1Δ (SZy1935), and rad52Δ (SZy1936) were grown at 23 °C in YPD medium, shifted to 37 °C for 2 h to inactivate condensin, and then treated in the absence or presence of 200 nm rapamycin for 6 h at 37 °C. The cells were then fixed for IF analysis. B, deletion of RAD52, but not FOB1, blocks the nucleolar fragmentation phenotype in the ycs4-2 condensin mutant. Images show the IF results from the experiment described in A. Nucleolar structure is shown in light gray, and the nucleus is shown in dark gray. C, quantification of cells showing fragmented nucleolus from the experiment in A (n > 100).
mycin (Fig. 1A). Under conditions when the ERC level was high, there was also an increased nucleolar fragmentation (Fig. 1, B–E). Because rapamycin causes condensin loading to rDNA and thereby prevents nucleolar fragmentation in rpa190-1 cells (14), we treated these cells with rapamycin and detected ERC levels. Indeed, rapamycin blocked ERC formation in rpa190-1 cells (Fig. 1A, compare lanes 7 and 8). These data show that ERC formation is closely correlated with nucleolar fragmentation, confirming our earlier observations (14). Because of the ease in detection of nucleolar fragmentation, we chose nucleolar staining to monitor ERC formation in our subsequent experiments.

To investigate whether Fob1 and Rad52 are involved in ERC formation under the aforementioned conditions, we generated rpa190-1 fob1/H9004 and rpa190-1 rad52/H9004 double mutants. In the absence of Fob1, inactivation of rpa190-1 still led to a high level of nucleolar fragmentation (Fig. 1, B and C). In contrast, the ERC level was reduced to the basal level after RAD52 was deleted (Fig. 1, B and C). To confirm that this ERC-suppressive effect is due to the Rad52-null mutation, we introduced a plasmid-borne RAD52-HA into the rpa190-1 rad52/H9004 mutant strain. In the presence of RAD52-HA, but not a control plasmid, nucleolar fragmentation was restored (Fig. 1, D and E). These results indicate that Rad52 is required for nucleolar fragmentation. To further verify this observation, we deleted RAD52 or FOB1 in the background of ycs4-2, a temperature-sensitive condensin mutant. In the ycs4-2 strain, rapamycin treatment does not lead to rDNA condensation due to lack of a functional condensin, resulting in elevated ERC levels (Fig. 2) (14). Consistently, we observed that deletion of RAD52, but not FOB1, in the ycs4-2 background suppressed the fragmented nucleolar phenotype (Fig. 2). Together, these results show that Rad52, but not Fob1, is

**FIGURE 3.** Rad52 becomes localized in the nucleolus in response to Pol I inactivation by the rpa190-1 mutation. A, inactivation of Pol I by rpa190-1 mutation leads to Rad52 localization with the nucleolus. Log phase cells carrying an empty vector pRS314 (SZy1883), pRS314(RAD52-HA) in WT RPA190 (SZy1881), and rpa190-1 (SZy1887) backgrounds were shifted to 23 °C or 37 °C for 1 h, and the localization of Rad52-HA was analyzed by IF using an anti-HA antibody. The nucleolus and the nucleus were visualized by anti-Nop1 antibody and DAPI staining, respectively. Co-localization of Rad52-HA (light gray) and the nucleolus (dark gray) are represented by white color in the merge images. B, shown is a kinetics analysis of Rad52 dynamics upon Pol I inactivation. The upper panel shows a representative cell image of Rad52-HA co-localized with the nucleolus (top) or excluded from the nucleolus (bottom). The Nop1-stained nucleolus is outlined by a white dotted line. The lower panel shows the percentage of cells with Rad52-HA co-localized with the nucleolus at different time points after shifting the temperature to 37 °C.
Rad52 Translocates into the Nucleolus upon rDNA Transcription Inhibition in Condensin Mutant—In WT cells, repression of rDNA transcription by rapamycin or nutrient starvation is accompanied by condensin-mediated rDNA condensation (14). We hypothesized that such rDNA compaction is important to protect rDNA integrity by keeping Rad52 out of the nucleolus. To test this, we inactivated condensin by shifting ycs4-2 strain to nonpermissive temperature, followed by treating these cells with rapamycin to inhibit rDNA transcription and then analyzed Rad52 localization (Fig. 5A, upper panel). In ycs4-2 cells at the permissive temperature or WT cells at both permissive and restrictive temperatures, rapamycin caused rDNA condensation, as evidenced by nucleolar contraction (14) (Fig. 5A, lower panel). In these cells, Rad52 remained to be excluded from the nucleolus (Fig. 5, A and B). In contrast, after inactivation of condensin in ycs4-2 strain at the restrictive temperature, repression of rDNA transcription by rapamycin caused entry of Rad52 into the nucleolus (Fig. 5, A and B). We further found that nutrient starvation had a similar effect on Rad52 localization (Fig. 5, C and D). These results indicate that condensin-mediated rDNA condensation prevents the entry of Rad52 into the nucleolus and Rad52-dependent homologous recombination.

Rad52-mediated rDNA Instability Compromises Cell Viability—To determine the physiological consequence of Rad52 along with the invasion of the nucleolus and subsequent rDNA instability, we performed cell viability assays in ycs4-2 and rpa190-1 strains with elevated rDNA instability. Viability was assessed by the colony formation assay. As shown in Fig.

rDNA transcriptional repression in the absence of condensation elicits Rad52 invasion of the nucleolus.

The fact that Rad52 is localized to the nucleolus suggests that it causes rDNA homologous recombination. To test this idea, we performed ChIP to detect the association of Rad52-HA with rDNA chromatin. In WT cells, Rad52-HA was undetectable at rDNA (Fig. 4A). In contrast, inactivation of rpa190-1 led to loading of Rad52-HA to rDNA chromatin (Fig. 4A). The association of Rad52-HA with rDNA does not appear to be sequence-specific, as Rad52 was detected throughout the entire rDNA repeat (data not shown), and the total Rad52-HA protein level remained relatively constant during the experiment (Fig. 4B).

Rad52 Translocates into the Nucleolus and Associates with rDNA Chromatin in Response to Pol I Inactivation—The observation that Rad52 is required for rDNA instability is interesting because Rad52 is better known for repair of rDNA lesions (28). It has been shown that Rad52 is normally localized in the nucleus but is excluded from the nucleolus (28). However, rDNA lesion undergoes a dynamic, transient exit from the nucleolus to get access to the Rad52-dependent HR machinery for repair (28). To gain insight into the mechanism of Rad52-dependent rDNA instability, we examined Rad52 localization. In agreement with the earlier study, Rad52 was localized in the nucleoplasm but excluded from the nucleolus under the normal growth condition (Fig. 3) in both wild type (WT) and rpa190-1 strains at the permissive temperature (23 °C). Upon shifting to the nonpermissive temperature, rDNA transcription was inhibited, but rDNA remained uncondensed in the rpa190-1 mutant (14). This condition caused Rad52 proteins to translocate into the nucleolus (Fig. 3). This observation indicates that required for hyper-recombination in transcriptionally repressed yet uncondensed rDNA array.

Rad52 Translocates into the Nucleolus and Associates with rDNA Chromatin in Response to Pol I Inactivation—The observation that Rad52 is required for rDNA instability is interesting because Rad52 is better known for repair of rDNA lesions (28). It has been shown that Rad52 is normally localized in the nucleus but is excluded from the nucleolus (28). However, rDNA lesion undergoes a dynamic, transient exit from the nucleolus to get access to the Rad52-dependent HR machinery for repair (28). To gain insight into the mechanism of Rad52-dependent rDNA instability, we examined Rad52 localization. In agreement with the earlier study, Rad52 was localized in the nucleoplasm but excluded from the nucleolus under the normal growth condition (Fig. 3) in both wild type (WT) and rpa190-1 strains at the permissive temperature (23 °C). Upon shifting to the nonpermissive temperature, rDNA transcription was inhibited, but rDNA remained uncondensed in the rpa190-1 mutant (14). This condition caused Rad52 proteins to translocate into the nucleolus (Fig. 3). This observation indicates that
FIGURE 5. Condensation of rDNA is necessary to prevent Rad52 localization to the nucleolus during rapamycin treatment or nutrient starvation. 

A, upper panel, shown is a schematic representation of the experimental procedure. Lower panel, early log phase cells of WT (SZy1884) and ycs4-2 (SZy1877) strains were shifted to 37 °C for 2 h to inactivate condensin and then treated with or without 200 nM rapamycin at 37 °C to inhibit rDNA transcription. Samples were harvested at different times for IF analysis. Localization of Rad52-HA was analyzed by IF using an anti-HA antibody. The nucleolus and the nucleus were visualized by anti-Nop1 antibody and DAPI staining, respectively. Co-localization of Rad52-HA (light gray) and the nucleolus (dark gray) are indicated by the white color in the merge images. The Nop1-stained nucleolus is outlined by a white dotted line. 

B, shown is the quantification of the data from A. -Rap, no rapamycin treatment; +Rap, rapamycin treatment. 

C, upper panel, a schematic representation of the experimental procedure is shown. Lower panel, early log phase cells of WT (SZy1884) and ycs4-2 (SZy1877) strains were shifted to 37 °C for 2 h to inactivate condensin, washed twice with water, and then incubated in synthetic complete tryptophan drop-out medium (+ Nutrient) or yeast nitrogen base solution (- Nutrient) at 37 °C to inhibit rDNA transcription. Samples were harvested at different times for IF analysis as described in A. D, the quantification of the data from C is shown. +N, complete medium; -N, nutrient starvation.
**Regulation of rDNA Stability**

**C**

Early log cells 23 °C → To inactivate condensin 37 °C, 2h → To inhibit rDNA transcription +/- Nutrient, 0, 30, 60 min at 37 °C → Fix

|            | WT | ycs4-2 |
|------------|----|--------|
| Nucleolus  | ![Image](image1.png) | ![Image](image2.png) |
| Rad52      | ![Image](image3.png) | ![Image](image4.png) |
| Merge      | ![Image](image5.png) | ![Image](image6.png) |
| Nucleus    | ![Image](image7.png) | ![Image](image8.png) |

- **23 °C**
- **0 min**
- **+ Nutrient 37 °C**
- **- Nutrient 37 °C**

|            | WT | ycs4-2 |
|------------|----|--------|
| Nucleolus  | ![Image](image9.png) | ![Image](image10.png) |
| Rad52      | ![Image](image11.png) | ![Image](image12.png) |
| Merge      | ![Image](image13.png) | ![Image](image14.png) |
| Nucleus    | ![Image](image15.png) | ![Image](image16.png) |

- **30 min**
- **60 min**

**D**

% cells with Rad52 colocalized with the nucleolus

- **WT + N**
- **WT - N**
- **ycs4-2 + N**
- **ycs4-2 - N**

**Time (min)**

**FIGURE 5—continued**
Rad52 Epistasis Genes Are Required for the rDNA Instability through Homologous Recombination—Rad52 is required for HR events as well as single-strand annealing and gene conversion (25). Rad52-mediated homologous recombination involves other Rad52 epistasis genes, including the Rad51 family (Rad51, Rad54, and Rad55), Rad59, and the Mre11 family (e.g. Xrs2) (25). To further characterize the HR machinery responsible for rDNA instability, we deleted representative genes from each group in the rad52Δ strain at a restrictive temperature, which was suppressed by RADS52 deletion. Similarly, rpa190-1 cells showed Rad52-dependent cell death when Pol I was inactivated (Fig. 6B). These results demonstrate that Rad52-dependent rDNA instability is a catastrophic event.

Rad52 Epistasis Genes Are Required for the rDNA Instability through Homologous Recombination—Rad52 is required for HR events as well as single-strand annealing and gene conversion (25). Rad52-mediated homologous recombination involves other Rad52 epistasis genes, including the Rad51 family (Rad51, Rad54, and Rad55), Rad59, and the Mre11 family (e.g. Xrs2) (25). To further characterize the HR machinery responsible for rDNA instability, we deleted representative genes from each group in the rpa190-1 background and investigated the effect on rDNA instability. As shown in Fig. 7, rad51Δ, rad54Δ, and rad55Δ mutations also suppressed the rDNA instability phenotype, although to a lesser extent than rad52Δ. This is likely due to the functional redundancy among genes within each subfamily. Rad59 is known to function specifically in intrachromosomal recombination (29). The rad59Δ phenotype further supports the role of intrachromosomal recombination in rDNA instability. rad51Δ suppressed rDNA instability phenotype less well than rad52Δ, indicating that both Rad51-dependent and Rad51-independent pathways are involved in rDNA instability. In contrast, deletion of XRS2 did not block rDNA instability (Fig. 7). Because Xrs2 is mainly responsible for sister chromatid homologous recombination (25), our results suggest that intrachromosomal recombination but not sister chromatid recombination mediates rDNA instability under the studied conditions. In addition, the yuk70Δ mutation did not affect rDNA instability, indicating that non-homologous end joining is not involved. Taken together, these results suggest that the Rad51 subfamily and Rad59 HR proteins are involved in Rad52-dependent intrachromosomal recombination of rDNA.

DISCUSSION

In the present study, we show that when rDNA transcription is repressed in the absence of proper condensin-mediated rDNA condensation, Rad52 readily enters the nucleolus, leading to rDNA instability. Together with our previous observations, these results indicate an antagonistic role of condensin and Rad52 in the maintenance of rDNA stability. (For a model, see Fig. 8.) Rad52 is a key component of the HR machinery involved in error-free DNA repair and genomic maintenance, including the rDNA region (25). In contrast to other genomic regions, Rad52-dependent DSB repair of the highly repetitive rDNA array is regulated differently. Rad52 is normally excluded from the nucleolus in both yeast and mammals (this study) (28, 30). Interestingly, the DSB region of rDNA exits transiently from the nucleolus, gaining access to Rad52 for HR-mediated repair (28). These observations suggest that nucleolar localization of Rad52 is deleterious and thus its accessibility to the nucleolus is restricted. Several studies have shown that Rad52 is required for homologous recombination of the rDNA array. Rad52 is responsible for the expansion of rDNA copy number by stimulating unequal sister chromatid recombination and spontaneous as well as aging-induced ERC formation by intrachromosomal recombination (7, 26, 27). Our study further demonstrates that Rad52 is required for nucleolar fragmentation and ERC formation when Pol I is repressed without proper rDNA condensation. Our study also provides insight into the mechanisms that prevent the invasion of Rad52 to the nucleolus to maintain rDNA stability. The accessibility of Rad52 to the nucleolus is restricted by active Pol I-dependent rDNA transcription during normal growth and condensin-mediated rDNA condensation during starvation conditions. Currently, the detail mechanism that causes Rad52 invasion to the rDNA array upon inhibition of rDNA transcription is still unknown. Our results suggest that actively transcribing rDNA has an intrinsic role in the maintenance of rDNA stability by keeping Rad52 off the nucleolus.

It has been reported that inactivation of Rad52 small ubiquitin-like modifier modification or the Smc5-Smc6 complex causes rDNA hyper-recombination and accumulation of ERC in budding yeast (28). In agreement with this finding, we demonstrated that invasion of Rad52 to the nucleolus causes uncon-
trolled HR and subsequent ERC formation. Although it remains to be determined whether Pol I-dependent rDNA transcription has a direct role in regulating Smc5-Smc6 activity or Rad52 small ubiquitin-like modifier modification, a simple explanation of our observation is that transcriptionally inactive rDNA in an uncondensed state may provide an “open” chromatin structure that is susceptible to Rad52 binding. Once in the nucleolus, Rad52 apparently mediates inappropriate HR among the highly repetitive homologous sequences of rDNA repeats, leading to elevated ERC formation and cell death. These catastrophic events are prevented in WT strains because rDNA transcriptional repression during starvation is always accompanied by condensin-mediated rDNA compaction (Fig. 8).

Our study indicates that the recruitment of Rad52 to rDNA precedes the formation of ERC and a fragmented nucleolus, suggesting that Rad52 is the cause of rDNA instability by stimulating hyperhomologous recombination, leading to ERC formation. It has been reported that Rad52 and its epistasis groups are recruited to the damaged DNA in a stepwise, temporally distinct manner for the assembly of HR machinery. Specifically, Rad51 appears to associate with the DNA first, followed by Rad52, Rad55, and Rad54 (31). In addition, the Rad52-mediated HR requires multiple steps, including end resection, strand invasion, strand exchange and DNA synthesis, formation and movement of Holliday junction, and finally, resolution of the junction (32). By using a pair of LEU2 direct repeat and homothallic switching endonuclease induced deletion assays, it has been demonstrated that the Rad52-dependent intrachromosomal homologous recombination reaction is complete by −5 h after induction of DSB (33). Consistent with this kinetic study, we show that there is a similar length of a “latent period” between the time of Rad52 binding to rDNA and the detection of ERC and fragmented nucleolus. This may explain the early detection of Rad52 association with rDNA and late formation of ERC and fragmented nucleolus.

Sir2 is an NAD$^+$-dependent histone deacetylase responsible for silencing the Pol II-transcribed genes and suppression of both intra- and interchromosomal recombination events in rDNA (34, 35). Sir2-dependent rDNA silencing requires active Pol I-dependent transcription (36, 37) and a Pol II-dependent transcription from a noncoding bidirectional promoter within the rDNA spacer (8). Therefore, during active transcription under normal growth, Sir2 is required for the maintenance of rDNA stability. However, inhibition of Sir2 by splitomicin or by the sir2Δ mutation in young yeast cells does not cause signifi-

**FIGURE 7. Rad52 epistasis genes are required for the homologous recombination-induced rDNA instability.** A, the Rad51 family and Rad59, but not Xrs2 or Yku70, are required for HR-induced rDNA instability. Early log phase cells of the indicated strains, WT (NOY260), rpa190-1 (NOY259), rpa190-1 rad51Δ (SZy1892), rpa190-1 rad52Δ (SZy1821), rpa190-1 rad54Δ (SZy1903), rpa190-1 rad55Δ (SZy1900), rpa190-1 rad59Δ (SZy1894), rpa190-1 xrs2Δ (SZy1907), and rpa190-1 yku70Δ (SZy1905) were incubated in YPD medium at 23 °C or 37 °C for 5 h. Their nucleoli were analyzed by IF analysis with an anti-Nop1 antibody as described in Fig. 1. B, the percentage of cells with fragmented nucleolus from the experiment described in A.
FIGURE 8. A model for the antagonistic role of condensin and Rad52 in rDNA instability and ERC formation. See under “Discussion” for detail. Diamonds, condensins; bars, rDNA repeats; small dots, RNA Pol I machineries; large dots, Rad52 proteins; dark lines, genomic DNA; circles, ERCs.
cant ERC formation (data not shown), suggesting that there is a novel mechanism(s) for actively transcribed rDNA to prevent Rad52 invasion. In conclusion, our present study further supports the dual role of Rad52 in controlling rDNA stability. Under normal physiological conditions, it functions to maintain genomic stability through a controlled DSB repair mechanism outside the nucleolus. However, inappropriate Rad52 localization to the nucleolus causes catastrophic events, such as cellular lifespan and reduced viability by stimulating ERC formation.

Acknowledgments—We thank Miao Chen for constructing several RAD52-related deletion strains and other members of the Zheng laboratory for helpful discussions.

REFERENCES

1. Warner, J. R. (1999) Trends Biochem. Sci. 24, 437–440
2. Kobayashi, T. (2008) BioEssays 30, 267–272
3. Tsang, C. K., Wei, Y., and Zheng, X. F. (2007) Cell Cycle 6, 2213–2218
4. Kobayashi, T., Heck, D. J., Nomura, M., and Horiuchi, T. (1998) Genes Dev. 12, 3821–3830
5. Kim, R. A., and Wang, J. C. (1989) Cell 57, 975–985
6. Brewer, B. J., Lockshon, D., and Fangman, W. L. (1992) Cell 71, 267–276
7. Kobayashi, T., Horiuchi, T., Tongaonkar, P., Vu, L., and Nomura, M. (2004) Cell 117, 441–453
8. Kobayashi, T., and Hanley, A. R. D. (2005) Science 309, 1033–1042
9. Defossez, P. A., Prusty, R., Kaebelerin, M., Lin, S. I., Ferrigno, P., Silver, P., Keil, R. L., and Guarente, L. (1999) Mol. Cell 3, 447–455
10. Johnson, F. B., Marciniak, R. A., and Guarente, L. (1998) Cur. Opin. Cell Biol. 10, 332–338
11. Shiloh, Y., and Lehmann, A. R. (2004) Nat. Cell Biol. 6, 923–928
12. Smith, J., and Rothstein, R. (1999) Genetics 151, 447–458
13. Buck, S. W., Sandmeier, L., and Boeke, J. D. (1998) Genetics 149, 1205–1219
14. Buck, S. W., Sandmeier, L., and Smith, J. S. (2002) Cell 111, 1003–1014
15. Cioci, F., Vu, L., Eliasen, K., Oakes, M., Siddiqi, I. N., and Nomura, M. (2003) Mol. Cell 12, 135–145