Condensin suppresses recombination and regulates double-strand break processing at the repetitive ribosomal DNA array to ensure proper chromosome segregation during meiosis in budding yeast

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ABSTRACT During meiosis, homologues are linked by crossover, which is required for bipolar chromosome orientation before chromosome segregation at anaphase I. The repetitive ribosomal DNA (rDNA) array, however, undergoes little or no meiotic recombination. Hyperrecombination can cause chromosome missegregation and rDNA copy number instability. We report here that condensin, a conserved protein complex required for chromosome organization, regulates double-strand break (DSB) formation and repair at the rDNA gene cluster during meiosis in budding yeast. Condensin is highly enriched at the rDNA region during prophase I, released at the prophase I/metaphase I transition, and reassociates with rDNA before anaphase I onset. We show that condensin plays a dual role in maintaining rDNA stability: it suppresses the formation of Spo11-mediated rDNA breaks, and it promotes DSB processing to ensure proper chromosome segregation. Condensin is unnecessary for the export of rDNA breaks outside the nucleolus but required for timely repair of meiotic DSBs. Our work reveals that condensin coordinates meiotic recombination with chromosome segregation at the repetitive rDNA sequence, thereby maintaining genome integrity.

INTRODUCTION

During meiosis I, homologues separate, whereas sister chromatids are kept together until meiosis II. To achieve this unique pattern of chromosome segregation, homologues are linked by crossover, a prerequisite for the establishment of homologue biorientation on the spindle in most eukaryotes (Zickler and Kleckner, 1999; Gerton and Hawley, 2005). Meanwhile, certain regions of the genome, such as the repetitive DNA sequences, undergo little or no recombination (Petes, 1980; Gottlieb and Esposito, 1989; Pan et al., 2011). How meiotic recombination is differentially regulated and how it is coordinated with chromosome segregation both remain to be further elucidated.

The evolutionarily conserved endonuclease Spo11 mediates the formation of programmed DNA double-strand breaks (DSBs) and is responsible for most recombination events during meiosis (Bergerat et al., 1997; Keeney et al., 1997). Additional factors are required for targeting Spo11 to chromosomal locations; they generate DSBs in a biased manner, forming so-called DSB "hot spots" at certain regions and "cold spots" at others. In general, open chromatin regions are more accessible to the meiotic DSB machinery, whereas heterochromatin loci and regions that harbor repetitive DNA sequences are less prone to DSB formation (Kleckner, 2006; Pan et al., 2011). Inhibition of DSB formation, and therefore meiotic recombination, at the repetitive DNA sequences is essential for preventing copy-number instability at these loci during cell division.

One well-studied model of the repetitive DNA sequence is the ribosomal gene cluster (rDNA) in the budding yeast *Saccharomyces cerevisiae*. Yeast rDNA is located on the right arm of chromosome XII, forming ~150 copies of 9.1-kb tandem repeats that are highly homogeneous and represent ~10% of the yeast genome (Petes and

Botstein, 1977). Meiotic recombination at the rDNA, as assayed by gene conversion, is inert (Gottlieb and Esposito, 1989; San-Segundo and Roeder, 1999), because DSB formation is inhibited partially through Sir2-dependent heterochromatin formation (Gottlieb and Esposito, 1989). In addition, Pch2 suppresses DSB formation both within and at the boundaries of the rDNA array (Vader et al., 2011). High-resolution mapping of Spo11-mediated meiotic DSBs shows only 0.15% of total DSBs are localized to the rDNA (Pan et al., 2011). Currently unknown is whether Spo11 has only limited access to the rDNA during meiosis, or whether additional chromosomal factors, such as Sir2 and Pch2, actively regulate DSB formation and repair at the rDNA.

A class of conserved protein factors, including condensin, mediates the reversible assembly of the chromosome structure, which is required for proper chromosome condensation and segregation (Hirano, 2012). Condensin is a five-subunit protein complex conserved from yeast to human, and condensin-like complexes are found in prokaryotes (Hirano, 2005). In budding yeast, the condensin complex, which consists of Smc2, Smc4, Brn1, Ycg1, and Ycs4, remains in the nucleus and binds to chromosomes throughout the cell cycle (Freeman et al., 2000). At interphase, when rDNA is actively transcribed, yeast condensin is diffused throughout the nucleus; it then becomes concentrated right before mitosis by Cdc14-dependent transcriptional repression of rRNA genes and remains so throughout mitosis (Tomson et al., 2006; Bachellier-Bassi et al., 2008; Clemente-Blanco et al., 2009). Therefore Cdc14 is required for promoting condensin association with the rDNA gene cluster and proper segregation of this chromosome locus (D'Amours et al., 2004; Sullivan et al., 2004). In addition, condensin also localizes to the centromeres at metaphase and assists chromosome biorientation on the spindle (Brito et al., 2010; Tada et al., 2011; Verzijlbergen et al., 2014), further demonstrating that condensin is spatially and temporally controlled during the cell cycle.

During yeast meiosis, condensin is required for resolving recombination-dependent chromosome linkages (Yu and Koshland, 2003). Similarly, condensin mediates chromosome organization and crossover interference during meiosis in Caenorhabditis elegans (Chan et al., 2004; Mets and Meyer, 2009), in which each pair of bivalents is strictly confined to a single crossover event. These results support a role for condensin in the processing of Spo11-mediated DSBs during meiosis. Fission yeast condensin may play a role in unloading chromosomal components after DNA damage repair by reannealing DNA strands (Akai et al., 2011). In contrast, mammalian condensin is involved in single-strand break repair (Heale et al., 2006; Griese et al., 2010), and upon artificial DSB formation in somatic cells, condensin is not subject to recruitment at the DSB site, so condensin is perhaps not necessary for DSB repair in vertebrates (Kim et al., 2002). Therefore the condensin function in DNA break repair appears to be organism specific and remains to be determined.

Using the dynamics of yeast rDNA gene clusters as a robust readout of condensin activity, we report here that condensin regulates rDNA recombination and segregation during yeast meiosis. Our work also reveals that condensin is required both for suppressing DSB formation and for promoting DSB processing at the rDNA, two distinctive condensin functions that are essential for proper meiotic chromosome segregation.

RESULTS
Dynamic localization of condensin during meiosis I in budding yeast
We showed previously that condensin is enriched at the rDNA array during yeast meiosis (Yu and Koshland, 2003). To determine

condensin dynamics, we developed a live-cell fluorescence microscopy method to observe condensin and rDNA movement in yeast cells that were induced to undergo synchronous meiosis (Figure 1). Using green fluorescent protein (GFP)–tagged condensin subunits Brn1 and Ycg1 and a red fluorescent protein (RFP)–marked rDNA array (Li et al., 2011), we found that at the beginning of meiosis (~5 h before anaphase I onset), Brn1-GFP was highly concentrated at the rDNA gene cluster (Figure 1A). Brn1-GFP became dispersed during prophase I (~2 h before anaphase I), and its association with rDNA decreased precipitously at late prophase I. Brn1-GFP was undetectable at the rDNA at metaphase I (~40 min before anaphase I, Shirk et al., 2011), but it reassociated with the rDNA array upon anaphase I onset (Figure 1A). These observations suggest that condensin is subject to relocation inside the yeast nucleus during meiosis.

This dynamic localization of Brn1-GFP was confirmed in yeast cells that were staged at prophase I with ndt80Δ (Xu et al., 1995) and at metaphase I with P_{CLB2}-CDC20 (Lee and Amon, 2003; Figure 1B). We found that both Brn1-GFP and Ycg1-GFP were colocalized to the rDNA region in all cells arrested at prophase I; in contrast, at metaphase I, 95% of cells showed no detectable Brn1-GFP or Ycg1-GFP on rDNA (Figure 1B). We observed similar Brn1-GFP dynamics using Nop1 as a marker for the rDNA locus (Figure 1C). Furthermore, we found that the protein level of Brn1 remained relatively stable in arrested prophase I (ndt80Δ) and metaphase I (P_{CLB2}-CDC20) cells (Figure 1D). The similarity of Brn1 level in these cells supports the idea that Brn1 is removed from the rDNA array after prophase I and then is relocated to other regions of the yeast genome at metaphase I (Brito et al., 2010). We therefore conclude that association of condensin with the rDNA array is temporally regulated during meiosis in budding yeast.

Previous work revealed that Ndt80 activates the polo-like kinase Cdc5 in yeast and that Cdc5 phosphorylates condensin subunits (Matos et al., 2008; Sourirajan and Lichten, 2008; St-Pierre et al., 2009); we therefore hypothesized that Cdc5 regulates condensin release from the rDNA. We ectopically expressed CDC5 in ndt80Δ cells using the CUP1 inducible promoter (Shirk et al., 2011) and found that Brn1-GFP was no longer colocalized with rDNA in 78% of these cells (t = 9, Figure 1E). Therefore Cdc5 is sufficient for the release of meiotic condensin from rDNA. In the absence of Cdc5 (P_{CLB2}-CDC5), yeast cells were arrested at a metaphase I–like stage (Lee and Amon, 2003), but Brn1-GFP remained colocalized with rDNA in ~37% of these cells (t = 9, Figure 1E), suggesting that additional factor(s) also play a role in condensin removal from the rDNA.

Condensin suppresses rDNA homologue exchange during meiosis
Because meiotic recombination takes place at prophase I, the temporal enrichment of condensin at the rDNA led us to hypothesize that condensin regulates DSB formation at this locus. To test this hypothesis, we determined the gene conversion rate at the rDNA gene cluster using a URA3 marker incorporated at a single 25S rRNA gene on one of the homologues (Figure 2A). Yeast diploid cells were induced to undergo meiosis to produce haploid spores, which were enclosed in tetrads. Yeast tetrads were dissected, and spores were genotyped. In wild-type cells (n = 206), most tetrads had the URA3 marker segregated 2:2, and only 0.5% of tetrads showed rearrangement of the URA3 marker by gene conversion (Figure 2A). Rearranged URA3 markers showed essentially equal ratios of 1:3 and 3:1 (unpublished data). These data suggest that very little meiotic recombination occurred at the rDNA, consistent with previous findings (Gottlieb and Esposito, 1989; San-Segundo and Roeder, 1999). In
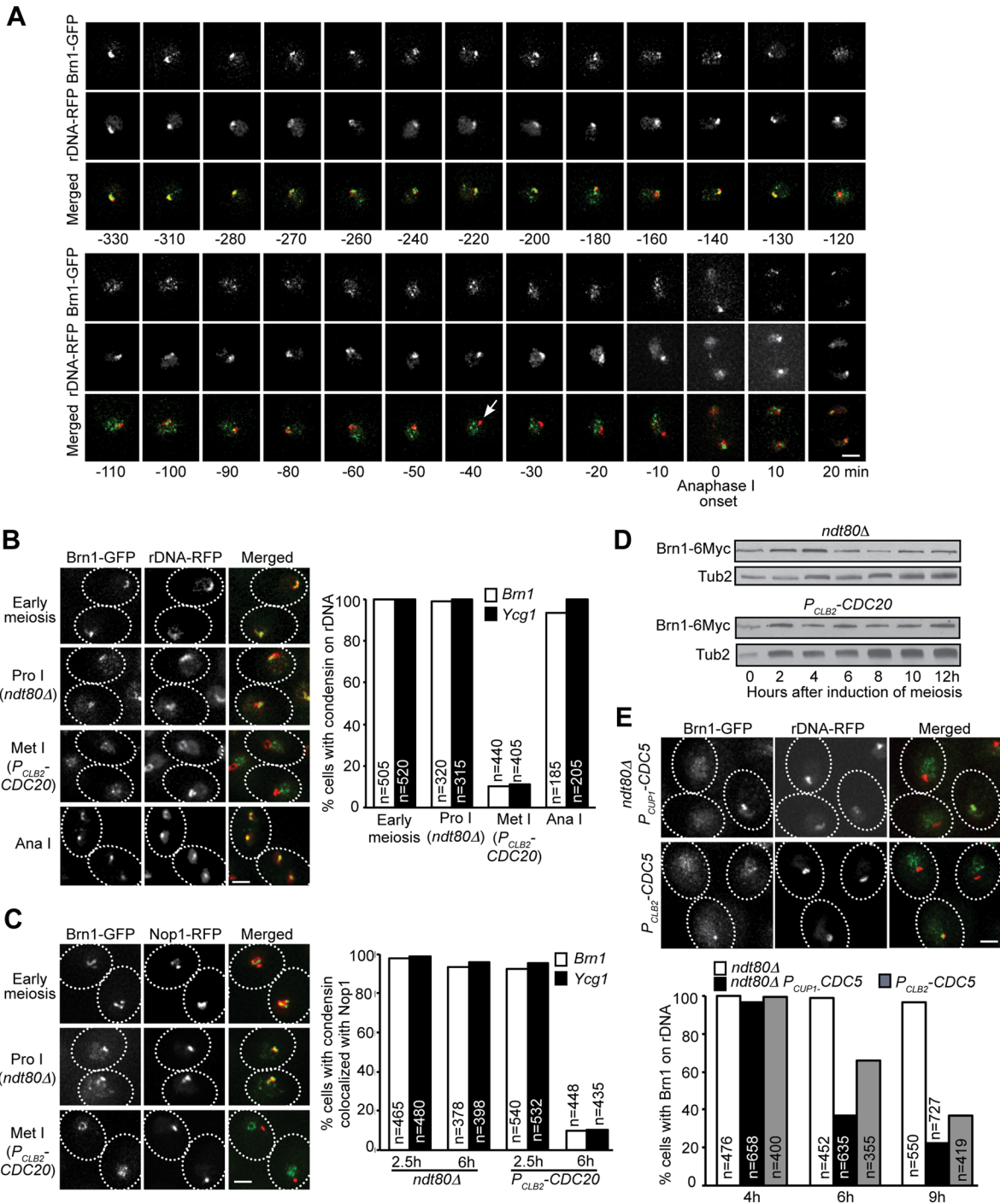
FIGURE 1: The sequestration and release cycle of condensin at the rDNA region during yeast meiosis. (A) Localization of condensin subunit Brn1 during yeast meiosis. Yeast cells (strain HY3528) were induced to undergo synchronous meiosis and then subjected to time-lapse live-cell fluorescence microscopy. Brn1 was tagged with GFP (green) and rDNA with tetO/tetR-RFP (red). Time zero was defined as the onset of anaphase I. Nuclear division shown by the residual tetR-RFP signal was used to determine the onset of anaphase I. Arrow indicates the point when condensin became undetectable at the rDNA region. (B) Representative images showing Brn1-GFP localization at early meiosis (strain HY3528), prophase I (Pro I, *ndt80Δ*, HY3584), metaphase I (Met I, P_{CLB2}-*CDC20*, HY3628), and anaphase I (AnaI, strain HY3528). Fluorescence microscopy was performed as in A. Note that Brn1-GFP overlaps with rDNA-RFP during early meiosis (~2.5 h after induction of meiosis), at prophase I (*ndt80Δ*), and at anaphase I, but not at metaphase I (P_{CLB2}-*CDC20*). Right, graph showing the quantitative analysis of Brn1-GFP and Ycg1-GFP localization to the rDNA region at the indicated stages. (C) Localization of Brn1-GFP and Nop1-RFP at the indicated cell cycle stages as in B. Right, graph showing quantitative analysis. Strains HY4143, HY4144, HY4145, and HY4146 were used. (D) Western blot showing the protein level of Brn-6Myc in *ndt80Δ* (HY4193) and P_{CLB2}-*CDC20* cells (HY4206). (E) Cdc5 regulates condensin release from rDNA. P_{CUP1}-*CDC5 ndt80Δ* (HY4008) and P_{CLB2}-*CDC5* (HY3995) cells were induced to undergo meiosis; 50 μM CuSO$_4$ was added to the culture medium 4 h after. Representative images are shown. Bottom graph, quantitative analysis of these cells. Bars, 2 μm.

rDNA

Genotype	GC rate (%)	Fold increase	Spore viability(%)
WT	0.5	1	98.1
ycs4-2	13.8	27.6	87.9
ycg1-2	7.8	15.6	95.2
smc2-8	3.8	7.6	89.3
sir2Δ	33.0	66.0	91.7

ARG4

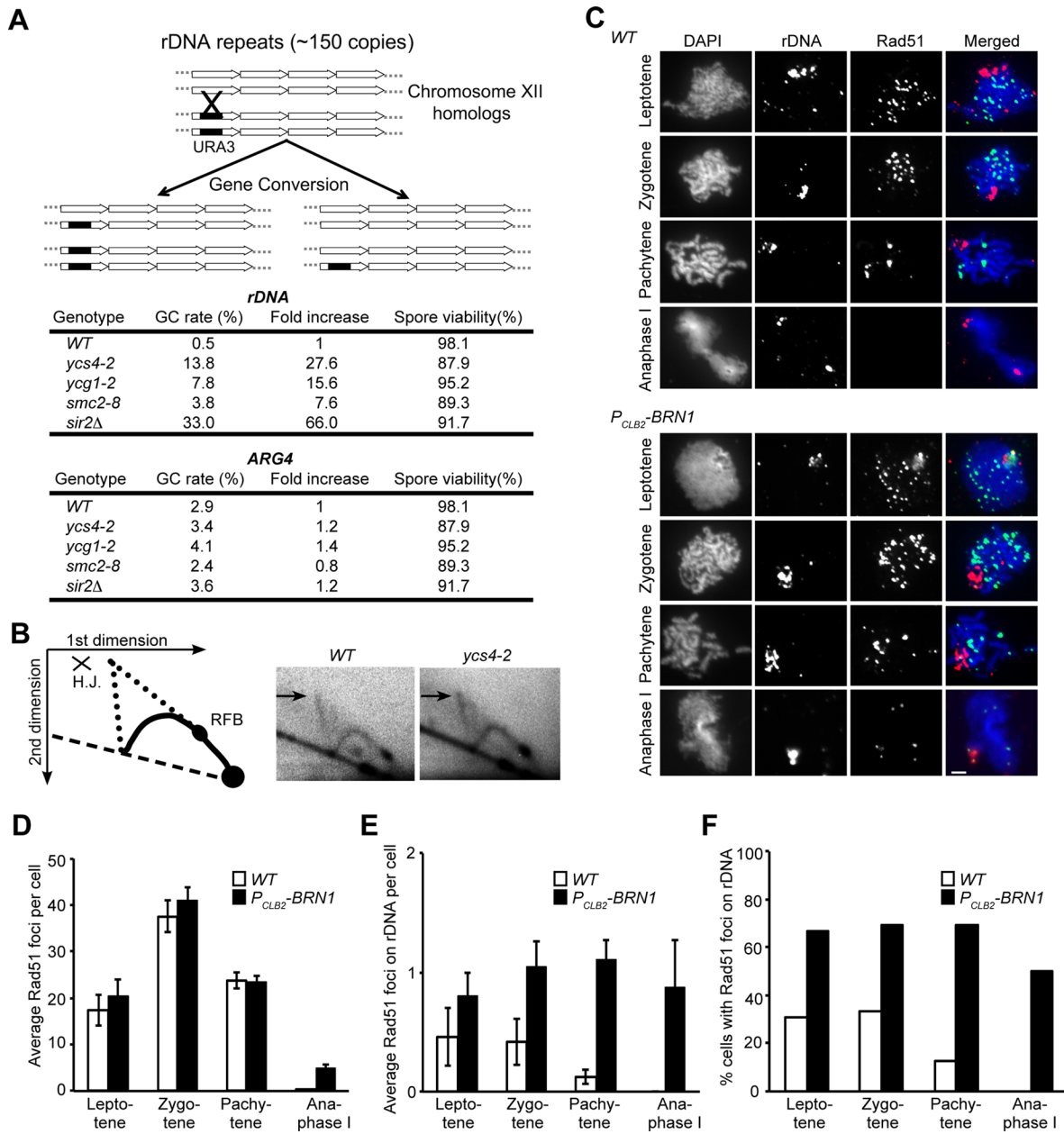| Genotype	GC rate (%)	Fold increase	Spore viability(%)
WT	2.9	1	98.1
ycs4-2	3.4	1.2	87.9
ycg1-2	4.1	1.4	95.2
smc2-8	2.4	0.8	89.3
sir2Δ	3.6	1.2	91.7

FIGURE 2: Requirement of condensin for suppressing rDNA recombination during yeast meiosis. (A) Meiotic gene conversion (GC) rate at rDNA and ARG4. Yeast strains (2998C, 2998, 3003, 3021, and 3025) were induced to undergo meiosis at 30°C, a semipermissive temperature for condensin mutants ycs4-2, ycg1-2, and smc2-8. Tetrads were dissected and genotyped at 25°C, and the GC rate was determined by marker segregation. Top, reporter assay of rDNA gene conversion. Note that an increased GC rate was observed in all condensin and sir2Δ mutants at rDNA but not at ARG4. (B) Two-dimensional gel mapping of Holliday junction (H.J.) molecules at rDNA. Left, schematic diagram showing the position of H.J. molecules. Arrows on the 2D gels point to the formation of H.J. molecules. RFB, replication fork barrier at the rDNA. (C) Rad51 focus formation during meiosis I. Wild-type (HY3140) and P_{CLB2}-BRN1 (HY2826) cells were induced to undergo synchronous meiosis. Aliquots were withdrawn at 3, 4, and 5 h after induction of meiosis. Nucleus surface spreads were performed, followed by indirect immunofluorescence. An anti-Rad51 polyclonal antibody was used to detect Rad51. Stages of meiosis were determined on the basis of chromosome morphology. Blue, 4′,6-diamidino-2-phenylindole–stained DNA; red, rDNA; green, Rad51. Bar, 2 μm. (D) Quantification of Rad51 focus formation in wild-type and P_{CLB2}-BRN1 cells during meiosis I. (E) Quantification of Rad51 focus formation at the rDNA locus in wild-type and P_{CLB2}-BRN1 cells. (F) The number of cells with Rad51 focus formation at the rDNA during meiosis I.

contrast, in the condensin mutant ycs4-2 (n = 202), the gene conversion rate increased ~28-fold at the semipermissive temperature of 30°C. Mutant spores showed similar ratios of 1:3 and 3:1 URA3 segregation, indicating gene conversion between rDNA homologues. Successful use of this assay required that temperature-sensitive con-

densin mutants be sporulated under semipermissive conditions, a state in which condensin function is only partially inactivated, resulting in the variation observed in condensin mutants ycs4-2, ycg1-2, and smc2-8 (Figure 2A). Similar to condensin mutants, sir2Δ cells showed a dramatic increase in rDNA gene conversion (Figure 2A).

Because the gene conversion rate at the rDNA was significantly increased in all condensin mutants tested (Figure 2A), and because condensin did not significantly alter the gene conversion rates at two nonrepetitive sites (ARG4 and HIS4) of the yeast genome (Figure 2A and unpublished data), we conclude that condensin is required for suppression of homologue exchange at the repetitive rDNA array.

Having established genetically that condensin represses homologue exchange, we next asked whether condensin is required for suppressing the formation of recombination intermediates at rDNA. The presence of Holliday junction molecules at the rDNA intergenic spacer in proliferating cells can be detected by two-dimensional (2D) gel electrophoresis (Brewer and Fangman, 1988). We applied this method to the analysis of rDNA recombination intermediates in yeast cells during meiosis. Four hours after induction of meiosis, when the majority of the cells were at prophase I, we harvested the cells, extracted chromosomal DNA, and prepared the samples for 2D gel analysis (Figure 2B). Holliday junction molecules were observed in both wild-type and condensin mutant ycs4-2, but mutant cells appeared to accumulate twice as many of these molecules, indicating that condensin regulates DSB formation and potentially DSB processing. One caveat is that we assayed only the intergenic rDNA spacer sequence, which may not be representative of the entire rDNA array.

We therefore used the formation of Rad51 foci as a cellular marker to probe the presence of meiotic DSBs. Yeast cells were induced to undergo meiosis, and nucleus surface spreads were prepared for immunofluorescence with a Rad51 antibody and a GFP antibody for rDNA-GFP (Figure 2C). At prophase I, which was divided on the basis of chromosome morphology into three substages (leptotene, zygotene, and pachytene), wild-type and P_{CLB2}-BRN1 cells showed similar total numbers of Rad51 foci (Figure 2D), suggesting that condensin is required for neither overall DSB formation nor regulation of DSB lifespan during meiosis, which is consistent with our previous findings (Yu and Koshland, 2003). We found that the rDNA region had little Rad51 focus formation in wild-type cells (Figure 2, E and F). In contrast, >60% of P_{CLB2}-BRN1 cells harbored at least one Rad51 focus at the rDNA at prophase I (Figure 2, E and F), and of note, Rad51 focus formation appeared to persist well into anaphase I in ~50% of the cells (Figure 2, C and F). These findings are consistent with the idea that condensin plays a dual role at the rDNA: it suppresses rDNA DSB formation, but it also acts in meiotic DSB repair.

Condensin is required for rDNA segregation during meiosis I

To determine how rDNA recombination affects chromosome segregation, we monitored the movement of the homologous rDNA gene clusters in live cells induced to undergo meiosis. At anaphase I, rDNA homologues formed an extended chromatin fiber ~6 min after the segregation of the bulk chromosomes, which were marked by RFP-tagged histone H2A (Supplemental Figure S1). At the end of meiosis I, the chromosome mass separated into two, associated with approximately equal amounts of rDNA-GFP, indicating that homologues completed their segregation (Supplemental Figure S1A). As in wild-type cells, rDNA homologues in the condensin mutant P_{CLB2}-BRN1 were localized primarily to the periphery of the chromosome mass before the onset of anaphase I (Supplemental Figure S1B), but in contrast to the wild type, rDNA homologues from mutant cells failed to form a chromatin fiber and never separated, whereas the rest of the chromosomes established a bilobed structure and apparently divided (Supplemental Figure S1B, t = 2 min). These results suggest that rDNA

homologues are entangled during meiosis and that Brn1 is required for homologue segregation.

We used the assembly and disassembly of spindle microtubules as an alternative means of further defining the timing of meiotic cell progression (Figure 3A). In wild-type cells, rDNA homologues formed a chromatin fiber along the length of the anaphase spindle (Figure 3A, t = 9–12 min), suggesting that homologous rDNAs are linked and that chromosome XII homologues, where rDNA is located, were pulled apart by forces from the spindle. After spindle disassembly, rDNA homologues segregated into opposite poles and formed two rDNA-GFP foci with similar fluorescence intensity (Figure 3A, t = 18–24 min). Of note, if one copy of the homologous rDNAs was marked, only one GFP focus appeared in one of the two poles, indicating that during meiosis I, homologous rDNAs segregated from each other (Supplemental Figure S1, C and D). In contrast to the wild-type cells, P_{CLB2}-BRN1 cells failed to segregate rDNA during the entire course of meiosis I (Figure 3A). To determine whether condensin subunits other than Brn1 are required for rDNA segregation, we observed rDNA dynamics in ycg1-2 cells that were induced to undergo meiosis at 34°C, a nonpermissive temperature for this allele (Yu and Koshland, 2003). In wild-type cells at that temperature, rDNA homologues segregated just as did those in the cell shown in Figure 3A, top. In contrast, ycg1-2 cells failed to segregate the rDNA homologues (Figure 3A). Therefore we conclude that condensin is required for proper rDNA segregation during yeast meiosis.

To address whether condensin plays a unique role at the rDNA gene cluster, we compared the segregation of GFP-marked chromosome loci from both chromosomes XII and IV (Yu and Koshland, 2003; Machin et al., 2005), the two largest chromosomes in yeast (Figure 3B). The majority of wild-type cells completed chromosome XII homologue segregation upon microtubule disassembly in meiosis I, as determined by GFP-marked loci at centromere XII, rDNA, and right telomere XII (Figure 3, D and F). Of note, a small portion of wild-type cells (~15%) appeared to separate marked rDNA only after spindle disassembly; these cells nevertheless generated viable spores (unpublished data). As expected, <5% of P_{CLB2}-BRN1 mutant cells segregated rDNA homologues throughout meiosis. In comparison, centromere XII– and telomere XII–marked loci achieved a higher degree of segregation in P_{CLB2}-BRN1 cells—85 and 62%, respectively (Figure 3, D and F)—suggesting that, in the absence of condensin function, chromosome XII homologues are more entangled at the rDNA region. Of note, GFP-marked telomere loci were disassociated at higher efficiency but failed to segregate to opposite spindle poles because of the linkage at the rDNA. In addition, we found that the segregation of chromosome IV homologues, assayed at three representative loci (centromere IV, right chromosome arm at LYS4, and right telomere IV), was less efficient in condensin-mutant cells (Figure 3E and unpublished data), which is consistent with previous observation that condensin is required for bulk chromosome segregation in yeast meiosis (Yu and Koshland, 2005). Similarly, we found that only 6% of ycg1-2 cells segregated homologous rDNA at the nonpermissive temperature, whereas the rate of centromere XII segregation was 86% (Figure 3F). In contrast, elimination of Sir2 and Pch2 only marginally altered rDNA segregation (Figure 3, G and H). These data confirm that condensin is essential for homologue segregation, in particular at the rDNA locus.

Cdc14 is required for condensin reloading at rDNA during anaphase I

Failure of rDNA homologue segregation in the absence of condensin function resembles that of the cdc14 mutants (Buonomo

et al., 2003; Marston *et al.*, 2003). To determine the dynamics of Cdc14 release from the meiotic yeast nucleolus, we performed live-cell microscopy to observe Cdc14-GFP, which served as the only source of Cdc14 in experimental cells (Figure 4). In wild-type cells before anaphase I onset, Cdc14-GFP formed an intense focus (Figure 4A), which was colocalized with a nucleolus marker Nop1 (unpublished data), indicating its association with the yeast nucleolus. The focused Cdc14-GFP signals disappeared at the onset of anaphase I ($t = 0$ min) and then recovered as two separated Cdc14-GFP foci, representing two divided nucleoli (Figure 4A). On average, the interval from the disappearance, and therefore release of Cdc14 from the nucleolus, to the reformation of Cdc14-GFP foci lasted 12.2 ± 0.6 min (SD, $n = 13$). In P_{CLB2}-*BRN1* cells upon anaphase I onset, the Cdc14-GFP focus disappeared on time, as judged by the elongation of the meiosis I spindle (Figure 4A). The average duration of Cdc14-GFP dispersal was 12.0 ± 0.5 min (SD, $n = 17$) in the mutant, not significantly different from that of the wild type. Of note, Cdc14-GFP recovered as a single GFP focus after meiosis I (P_{CLB2}-*BRN1*, $t = 12$ min), suggesting that the nucleolus failed to divide in P_{CLB2}-*BRN1* cells. This result supports our observation that rDNA homologues, which are tightly associated with the yeast nucleolus, remain linked in the absence of condensin function. We note that a small portion of Cdc14-GFP released from the nucleolus was concentrated at one of the spindle poles at anaphase I (wild type, $t = 12$, and P_{CLB2}-*BRN1*, $t = 9$ min, arrows). The biological significance of Cdc14's association with the spindle pole is unknown. Cdc14 release from the nucleolus and Cdc14 reloading appeared to be on time in P_{CLB2}-*BRN1* cells, because cells committed to anaphase I simultaneously with or without Brn1 (Figure 4B). These findings indicate that release of Cdc14 from the nucleolus is independent of condensin.

Previous work revealed that anaphase enrichment of condensin in the nucleolus depends on Cdc14, which is released from the rDNA by the FEAR network in vegetative yeast cells (D'Amours *et al.*, 2004); we therefore hypothesized that Cdc14 regulates condensin reload at the rDNA at anaphase I. We used the *cdc14-1* allele (Marston *et al.*, 2003) to inactivate Cdc14 and found that Brn1-GFP was no longer associated with the rDNA region upon the onset of anaphase I in >75% of cells observed (Figure 4C). Therefore our data show that Cdc14 is required for condensin reloading at the rDNA at anaphase I onset, but not vice versa.

Condensin inhibits Spo11-dependent homologue linkage formation at the rDNA

We hypothesized that abnormal meiotic recombination in condensin mutants leads to rDNA missegregation. To test this hypothesis, we observed rDNA homologue segregation in *spo11Δ* and *spo11Δ* P_{CLB2}-*BRN1* mutant cells (Figure 5). In the absence of Spo11, Rad51 focus formation, and therefore meiotic DSBs, was essentially eliminated (Supplemental Figure S2, A–C), and homologues did not form linkages and would segregate randomly (i.e., half the time homologues would segregate to opposite spindle poles, and the other half to the same pole; for a diagram, see Supplemental Figure S2D). This random segregation of homologues was indicated in cells with the GFP marker positioned at three tested loci in both homologues: centromere XII, rDNA, and telomere XII (Figure 5A). Without Spo11, P_{CLB2}-*BRN1* cells segregated rDNA homologues at a rate similar to that of the *spo11Δ* single mutant (Figure 5A), suggesting that rDNA homologue linkage formed in the absence of condensin depends on Spo11.

Next we asked whether the low rate of DSB formation at the rDNA array was due to a restraint on Spo11's access to the rDNA

region. We forced Spo11 localization to the rDNA with a P_{REC8}-*Spo11-tetR* fusion, in which the expression of Spo11–tet repressor (tetR) was under the control of the meiosis-specific *REC8* promoter (Figure 5B). P_{REC8}-*Spo11-tetR* fusion was directed to tet operator (tetO)–rDNA (Figure 5B). In wild-type cells, only minimal DSBs were observed at the rDNA region (Figures 2, E and F, and 5C); however, 60% of cells with the P_{REC8}-*Spo11-tetR* had at least one Rad51 focus at the rDNA region (Figure 5C), indicating that merely forcing localization of Spo11 was sufficient to generate DSBs at rDNA. Crucially, these Rad51 foci disappeared before cells entered into anaphase I, and rDNA homologues segregated properly (Figure 5, C and D), indicating that elevated DSB formation at the rDNA by itself does not lead to a detrimental effect on chromosome segregation. Of note, P_{REC8}-*Spo11-tetR* cells produced viable spores (Figure 5B), indicating that meiotic DSBs formed elsewhere in addition to the rDNA array. These results support the idea that condensin not only suppresses Spo11-dependent DSB formation, but, more important, it also regulates rDNA DSB repair.

Because high levels of RNA polymerase I (Pol I) transcription can stimulate rDNA recombination (Lin and Keil, 1991), and Pol I–mediated rRNA transcription is antagonistic to condensin activity at the rDNA gene cluster in vegetative yeast cells (Torres-Rosell *et al.*, 2007a; Johzuka and Horiuchi, 2009), we attempted to determine whether Pol I transcription other than Spo11 regulates rDNA recombination in yeast meiosis. We generated the P_{CLB2}-*AID-RPA43* allele to deplete RPA43, an essential subunit of Pol I, during meiosis (Supplemental Figure S3A). In the absence of Pol I activity, rDNA homologues segregated; in contrast, they remained linked in P_{CLB2}-*AID-RPA43* P_{CLB2}-*BRN1* double-mutant cells (Supplemental Figure S3B), suggesting that meiotic transcription at the rDNA does not play a significant role in rDNA dynamics. These results further support the idea that failure of rDNA homologue segregation in the absence of condensin is due to Spo11-mediated meiotic recombination.

Condensin is required for DSB processing in yeast meiosis

To address the timing of condensin function that is required for meiotic recombination and chromosome segregation, we determined the execution point of condensin using the *ycg1-2* allele (Lavoie *et al.*, 2004) because of its reversibility in inactivation and reactivation of the Ycg1 protein with temperature shift (Figure 6). We used the *GAL4.ER* P_{GAL}-*NDT80* allele (Carlile and Amon, 2008) to stage yeast cells at pachytene, which is permissible for DSB formation. On the addition of estradiol and activation of *NDT80* gene expression, yeast cells resumed meiosis (Figure 6A). We found that the total number of Rad51 foci formed was comparable in arrested yeast cells at pachytene, but >60% of *ycg1-2* cells showed Rad51 focus formation at the rDNA when meiosis was induced at the nonpermissive temperature (Figure 6, B–D). Furthermore, Rad51 foci persisted at the rDNA region at anaphase if *ycg1-2* cells were induced to undergo meiosis at the nonpermissive temperature (Figure 6, E and F). Similarly, ~50% of *ycg1-2* cells retained Rad51 foci at rDNA even at anaphase if these cells were shifted from the permissive to the nonpermissive temperature before being released from pachytene (Figure 6, E–G). In contrast, there was little Rad51 focus formation on anaphase chromosomes when cells were switched from the nonpermissive to the permissive temperature (Figure 6, E–G), demonstrating that upon the reactivation of Ycg1 at the permissive temperature, DSBs can be repaired.

To determine whether timely repair of rDNA breaks permits chromosome segregation during anaphase I, we observed rDNA
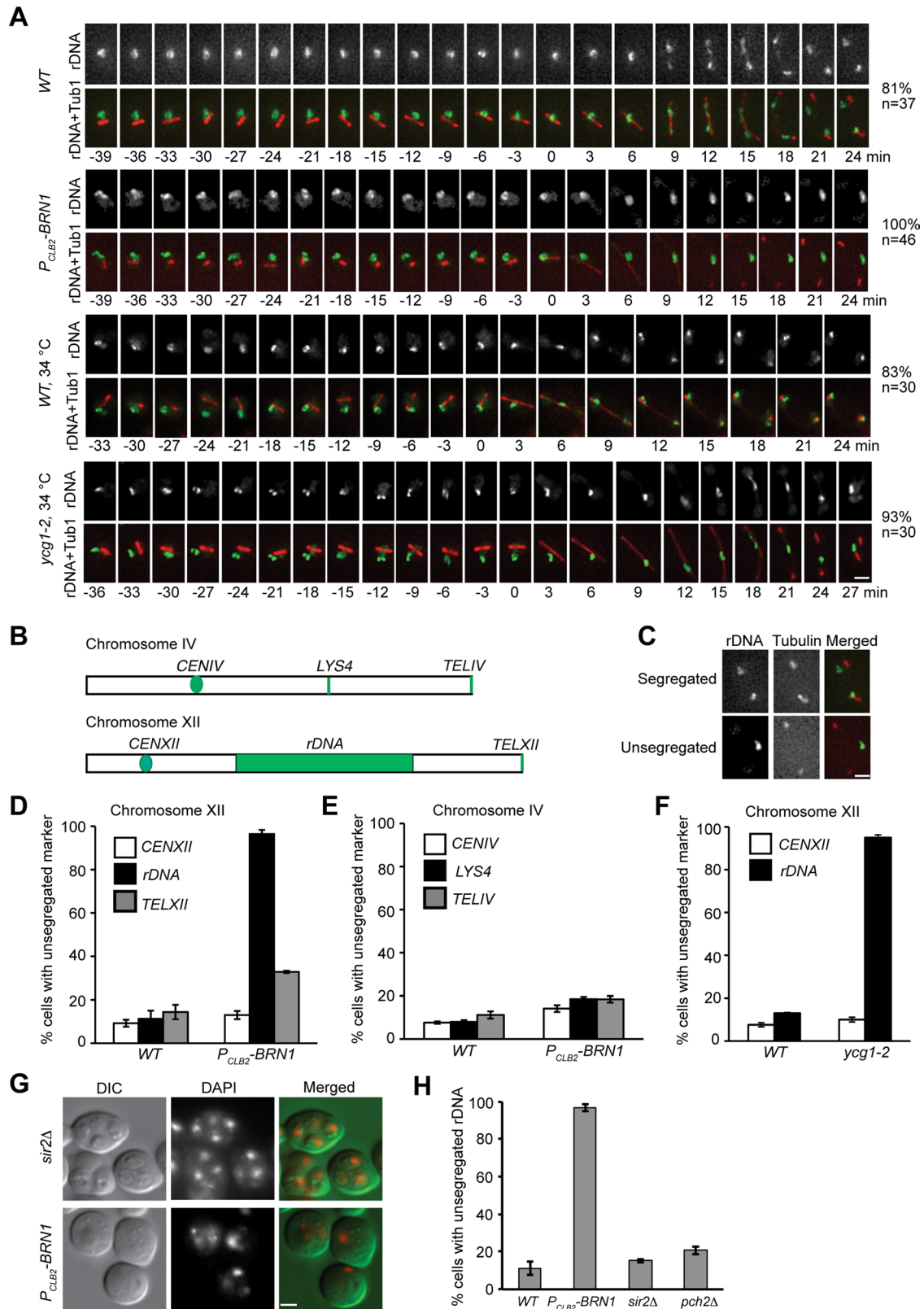
FIGURE 3: Requirement of condensin for rDNA segregation at anaphase I. (A) Live-cell microscopy showing rDNA dynamics in wild type (WT, HY1829) and condensin mutants, P_{CLB2}-BRN1 (HY2257) and ycg1-2 (HY2847). Both rDNA homologues are marked by tetO/tetR-GFP; α-tubulin (Tub1) was tagged with RFP. Time zero was defined as the onset of anaphase I when the spindle elongates. Microscopy temperature was set at 30°C for P_{CLB2}-BRN1. To inactivate ycg1-2, microscopy was performed at 34°C. (B) Schematic diagram showing the positions of GFP-marked loci on chromosomes IV and XII. (C) Representative images showing the segregation of GFP-marked rDNA during meiosis I. Segregation of GFP-marked loci in meiosis I was determined in cells upon spindle disassembly. (D) Segregation of
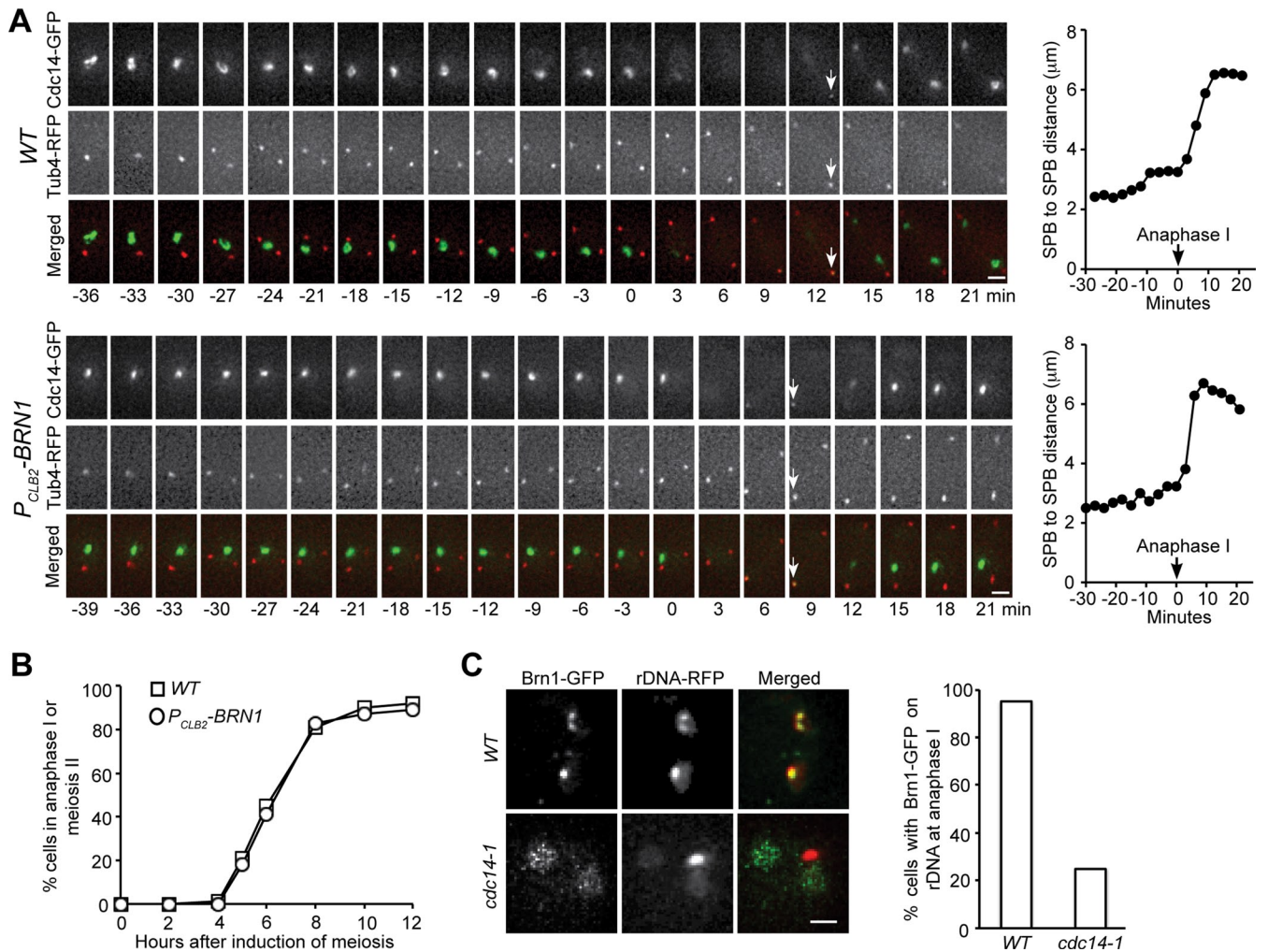
FIGURE 4: The relationship between Cdc14 release and condensin reloading at the rDNA. (A) Cdc14 dynamics in wild-type (HY2100) and P_{CLB2}-BRN1 (HY2894) cells during yeast meiosis. Yeast cells were prepared for time-lapse live-cell microscopy as shown in Figure 1A. Cdc14 was tagged with GFP; Tub4, the γ-tubulin in yeast, was tagged with RFP. Time zero was defined as the onset of anaphase I. Note that the Cdc14-GFP focus disappeared at the onset of anaphase I and reemerged as two separated foci after meiosis I in the wild-type cell, but in the mutant cell it reemerged as a single focus. Arrows indicate the localization of Cdc14-GFP to one of the spindle pole bodies during anaphase I. Right, graphs showing SPB-to-SPB distance during metaphase I and anaphase I. (B) Cell progression in wild type and P_{CLB2}-BRN1. Cell cycle stage was determined on the basis of spindle morphology. Both anaphase I and meiosis II cells are scored. (C) Reloading of Brn1-GFP at rDNA depends on Cdc14. Yeast cells (HY4056) were induced to undergo synchronous meiosis at 35°C, and time-lapse live-cell microscopy was performed at 35°C. Left, representative images. Right, quantitative analysis of Brn1-GFP localization. More than 200 cells were counted. Bars, 2 μm.

homologue segregation by live-cell fluorescence microscopy (Figure 6H). At the permissive temperature, rDNA homologues separated in *ycg1-2* cells, but at the nonpermissive temperature, they failed to do so (Figures 3A and 6H). If *ycg1-2* cells were induced to undergo meiosis but arrested at pachytene at the nonpermissive temperature and then were returned to the permissive temperature upon pachytene release, 78% of cells segregated rDNA-GFP at anaphase I (Figure 6H). In contrast, if *ycg1-2* cells were shifted from

the permissive temperature to the nonpermissive temperature after pachytene, only 40% could segregate rDNA (Figure 6H). Of importance, rDNA-GFP segregated in the *ycg1-2 spo11Δ* double-mutant cells at a similar ratio to those of *spo11Δ* cells when the double-mutant cells were released to the nonpermissive temperature after pachytene (Figure 6H). Together these data further demonstrate that condensin promotes rDNA break repair and is essential for proper chromosome segregation.

GFP-marked loci on chromosome XII (HY1829, HY2257, HY2804, HY2805, HY2810, and HY2813). Note that rDNA failed to segregate in P_{CLB2}-BRN1 cells. (E) Segregation of GFP-marked loci on chromosome IV during meiosis I (HY2852, HY2853, HY2858, HY2859, HY2864, and HY2865). (F) Segregation of rDNA and centromere XII in wild-type (HY2810) and *ycg1-2* cells during meiosis I (HY3116). (G) Representative images showing *sir2Δ* and P_{CLB2}-BRN1 cells during meiosis. (H) Segregation of rDNA in *sir2Δ* and *pch2Δ* cells during meiosis I (HY3544 and HY3524). For D–F and H, at least 200 cells with disassembled spindles were scored. Bars, 2 μm.
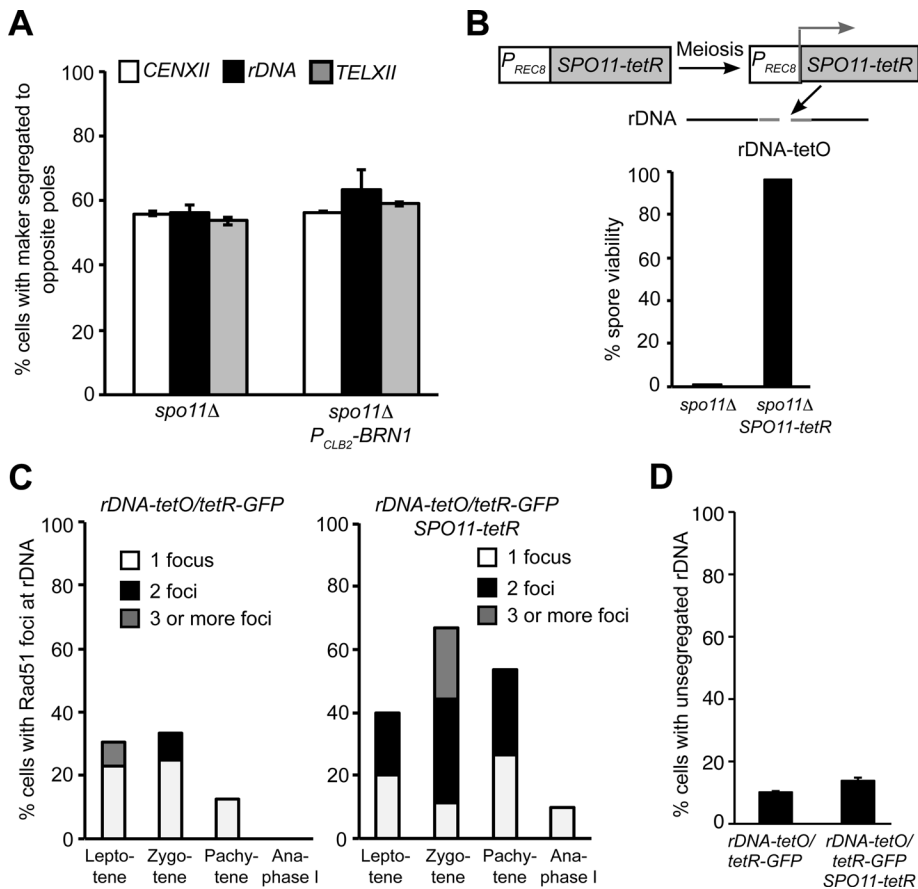
FIGURE 5: Condensin regulates rDNA recombination and segregation during yeast meiosis in a spo11-dependent manner. (A) Segregation of GFP-marked loci on chromosome XII in the absence of Spo11 during meiosis (HY2193, HY2255, HY2866, HY2895, HY2896, and HY3102). Note that in *spo11Δ* cells, homologues segregate randomly; for schematic diagrams, see Supplemental Figure 2D. (B) Schematic diagram showing the forced localization of Spo11 to the rDNA array. Note that *SPO11*-tetR suppresses the *spo11Δ* (HY2339 and HY3409) phenotype. (C) Forced localization of Spo11 generating DSBs at rDNA. Yeast cells (HY3159) were induced to undergo meiosis, and nucleus surface spreads were prepared for indirect immunofluorescence as in Figure 2C. Rad51 focus formation was determined and quantified. (D) Quantification of rDNA segregation during meiosis I. Both strains are marked with rDNA-tetO/tetR-GFP. More than 200 cells were counted.

Condensin is required for the timely repair of an artificial rDNA break in meiosis

To investigate further how condensin regulates DSB processing, we generated an artificial DSB at the rDNA using the I-*Sce*I system (Figure 7A). Artificial DSBs by the I-*Sce*I site-specific endonuclease appear to be processed as endogenous Spo11-mediated DSBs (Fukuda *et al.*, 2008) but would simplify our assay of condensin function in DSB repair. We abolished the formation of endogenous DSBs by the *spo11Δ* allele and then inserted one copy of the recognition sequence of I-*Sce*I into the 25S rRNA gene where the *URA3* marker was incorporated (Figure 2A). On the induction of I-*Sce*I by the meiosis-specific *REC8* promoter, I-*Sce*I cut its recognition sequence with ~80% efficiency, as determined by a PCR-based assay of chromosome breakage (Figure 7B). By fluorescence microscopy, we observed both Rad51 and Rad52 focus formation at the rDNA region (Figure 7C and Supplemental Figure S4, A–C), indicating the formation of rDNA breaks.

By time-lapse microscopy, we visualized the dynamics of I-*Sce*I–induced Rad52 focus at the rDNA gene cluster in live yeast cells

(Supplemental Figure S4D). The half-life of the Rad52 focus in *spo11Δ* cells was 20 min; in contrast, Rad52 foci lasted twice as long in *spo11Δ P_CLB2-BRN1* double-mutant cells (Figure 7D), suggesting that DSB repair, if it occurs, is delayed in the absence of condensin function. We found that ~48% of *spo11Δ P_CLB2-BRN1* cells with ectopic rDNA breaks carried Rad52 foci into anaphase I (Supplemental Figure S4E). This observation is consistent with the finding that ~50% of *P_CLB2-BRN1* and *ycg1-2* cells carried Rad51 foci at the rDNA during anaphase I (Figures 2F and 6G), confirming that condensin plays an active role in processing both endogenous and ectopic DSBs during yeast meiosis.

Earlier work showed that rDNA breaks formed in proliferating yeast cells are transiently transported outside the nucleolus before the breaks are repaired (Torres-Rosell *et al.*, 2007b). To determine whether meiotic rDNA breaks are repaired in a similar manner, we used Rad52-GFP to mark the formation of rDNA DSBs and Nop1-RFP for the nucleolus in yeast cells that were induced to undergo synchronous meiosis (Figure 7E). About 70% of cells positioned the Rad52-GFP focus outside the nucleolus in both *spo11Δ* and *spo11Δ P_CLB2-BRN1* cells (Figure 7E), indicating that meiotic rDNA breaks are relocated for repair but that condensin is dispensable for break export outside the nucleolus.

Ectopic production of I-*Sce*I DSBs at the rDNA did not exert an adverse effect on rDNA segregation in wild-type cells; indeed, ~70% of *spo11Δ* cells with induced rDNA DSBs properly segregated rDNA homologues to opposite poles (Figure 7F). This observation also indicates that artificially induced DSBs are sufficient for establishing a bipolar orientation of chromosome XII homologues (Figure 7F). In contrast, *spo11Δ P_CLB2-BRN1* double-mutant cells failed to separate rDNA in the presence of I-*Sce*I breaks (Figure 7F), further demonstrating that failure of DSB repair in the absence of condensin function causes chromosome missegregation.

DISCUSSION

We have shown here that during yeast meiosis, condensin is highly enriched at the rDNA array and regulates crossover between the repetitive rDNA sequences, thereby maintaining rDNA copy number stability and genome integrity. Not only is condensin required for suppressing DSB formation at the rDNA, it also promotes DSB processing, demonstrating that condensin is necessary for proper meiotic DSB repair. These observations are consistent with previous findings from both budding yeast and *C. elegans* that condensin plays an essential role in regulating meiotic recombination (Yu and Koshland, 2003; Chan *et al.*, 2004; Mets and Meyer, 2009); they also provide further insights into the mechanism by which condensin-mediated chromosome organization regulates meiotic chromosome dynamics.
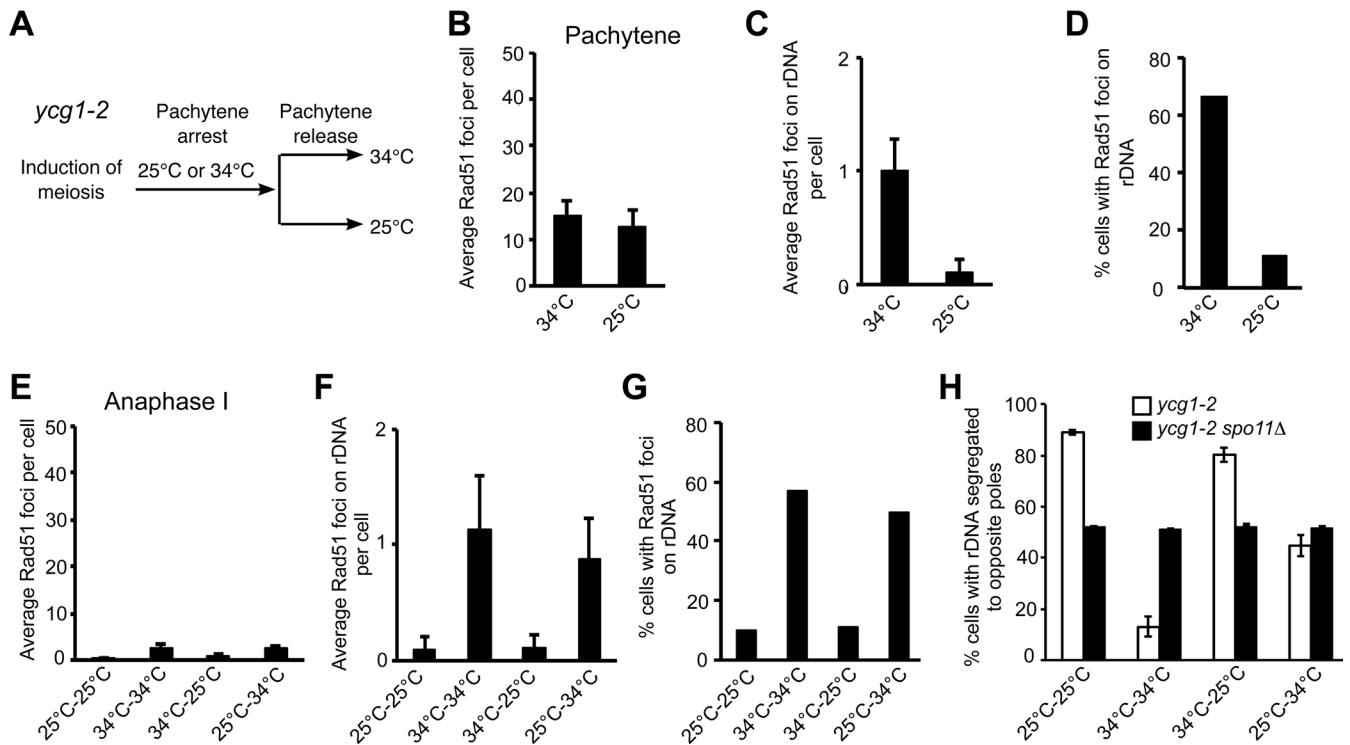
FIGURE 6: The execution point of Ycg1 at rDNA during meiosis. (A) A schematic diagram showing the experimental procedure. Yeast cells from the *ycg1-2* and *ycg1-2 spo11Δ* strains (HY3137 and HY4677) were induced to undergo synchronous meiosis and arrested at pachytene with *GAL4.ER P_GAL-NDT80*. On the addition of estradiol and production of Ndt80, *ycg1-2* cells were released from pachytene arrest. To inactivate the *ycg1-2* allele, we cultured yeast cells at 34°C. (B–D) Quantification of Rad51 focus formation at pachytene. Yeast cells were staged at pachytene at either 25 or 34°C for 6 h after meiotic induction. Rad51 focus was determined by nucleus surface spreads and indirect immunofluorescence as in Figure 2C. (E–G) Quantification of Rad51 focus formation after pachytene release. We added 1 μM estradiol to release the cells from pachytene arrest. Samples were collected 1 h after addition of estradiol, followed by immunofluorescence microscopy. (H) Quantification of rDNA segregation during meiosis I. Yeast cells were staged at pachytene at either 25°C (permissive temperature) or 34°C (restrictive temperature) and then switched to 34 or 25°C upon the addition of estradiol. Segregation of GFP-marked rDNA was determined as shown in Figure 3C.

Condensin is highly enriched at the yeast rDNA gene cluster at the meiotic prophase I. It is released from the rDNA at the prophase I/metaphase I transition and then is reloaded at anaphase I during chromosome segregation. Release of rDNA-associated condensin is regulated by Cdc5, whereas reloading of condensin at anaphase I requires Cdc14 (Figure 8), which itself resides at the rDNA/nucleolus during much of the cell cycle (Shou *et al.*, 1999; Visintin *et al.*, 1999). Subunits of the monopolin complex are required for condensin recruitment to rDNA in proliferating yeast cells (Johzuka and Horiuchi, 2009) and Cdc5 is required for monopolin relocalization (Clyne *et al.*, 2003), indicating that Cdc5 may indirectly regulate condensin release from rDNA during meiosis. Regulation of condensin relocalization by the kinase (Cdc5) and phosphatase (Cdc14) pair highlights the importance of posttranslational modification of condensin subunits.

In the absence of condensin, excessive DSBs form at the rDNA gene cluster, leading to unresolvable homologue entanglement (Figure 8). There is no evidence to indicate that condensin physically interacts with Spo11, but forced localization of Spo11 to the rDNA gene cluster appears to be sufficient to generate rDNA DSBs (this study), suggesting that condensin generates an inhibitory signal that limits Spo11's access to the rDNA region. Alternatively, inclusion of rDNA within the nucleolus may shield the rDNA from the recombination machinery during meiosis. These two possibilities are not mutually exclusive. We found that the rDNA gene array

remains very dynamic and primarily localized to the periphery of the yeast nucleus in condensin mutants (unpublished data), suggesting that the nuclear territory that the rDNA gene cluster occupies is less critical for meiotic recombination. It is intriguing that another SMC complex, cohesin, plays a similar role in regulating rDNA homologue segregation (Yu and Koshland, 2005). Unlike in mitosis, however, during yeast meiosis, cohesin does not form cohesin-associated regions (CARs) at the rDNA region, although cohesin remains associated with the rDNA as assayed by chromatin spread (unpublished data). The potential interplay of meiotic condensin and cohesin at the rDNA region remains to be determined. In addition, condensin can also modulate rDNA topology in proliferating yeast cells (D'Ambrosio *et al.*, 2008). Therefore the possibility also exists that condensin-mediated rDNA topology could be restrictive to Spo11-dependent DSB formation at this locus.

Our condensin execution-point experiment reveals that during yeast meiosis, condensin promotes DSB processing at the rDNA gene cluster. This reasoning is further supported by the observation that upon DSB formation, both Rad51 and Rad52 foci persist on rDNA at anaphase I in condensin mutants. Such findings are consistent with earlier observations that condensin is required for the disassembly of the synaptonemal complex and crossover interference in yeast and *C. elegans*, respectively (Yu and Koshland, 2003; Chan *et al.*, 2004; Mets and Meyer, 2009). Converging evidence shows

that condensin may play a conserved role in DSB processing in both meiosis and mitosis. In humans, condensin is essential for DSB repair mediated by the homologous recombination pathway (Wood et al., 2008). The condensin-like complex MukB forms aggregates that interact and bring distant DNA ends together in *Bacillus subtilis* (Gruber and Errington, 2009). Similarly, yeast condensin, much like the related cohesin complex, acts like the molecular glue that engages sister chromatids at the rDNA gene cluster in proliferating cells (Lavoie et al., 2004; Ide et al., 2010). These findings indicate that condensin acts early in the DSB repair pathway, for example, in determining the preferred DSB repair template: sister chromatid versus homologue. Our finding of an increased rDNA gene conversion rate in condensin mutants supports this idea.

In proliferating yeast cells, DSBs formed at the rDNA gene cluster are transported outside the nucleolus region for further processing (Torres-Rosell et al., 2007b). Using the I-SceI break, we found that meiotic DSBs formed at the rDNA appear to have a similar fate to the mitotic ones, but condensin is dispensable for exporting the broken chromosome ends outside the nucleolus. In the absence of condensin function, I-SceI breaks are not repaired in a timely manner, indicating another possibility: condensin also acts at a late stage of the recombination pathway—for example, through DNA ligase IV, which has been shown to interact physically with condensin in mammalian cells (Przewloka et al., 2003).

Condensin is enriched at the rDNA but is also distributed along the pachytene chromosomes during meiosis in budding yeast (Yu and Koshland, 2003). The dramatic defect in rDNA segregation observed in condensin mutants is perhaps proportional to the amount of condensin associated with this locus. Alternatively, condensin may play a rather unique role in yeast rDNA recombination and segregation. In vegetative yeast cells, condensin loading to the rDNA requires the attenuation of Pol I activity in rRNA transcription at anaphase (Clemente-Blanco et al., 2009). Because yeast meiosis takes place in a starved condition, Pol I activity is already decreased throughout meiosis. Indeed, inactivation of Pol I in yeast meiosis has little effect on rDNA segregation (this study). As in condensin mutants, failure of rDNA segregation is also observed in cells lacking Cdc14 activity during meiosis (Buonomo et al., 2003; Marston et al., 2003). In these cells, spindles fail to disassemble, which blocks the cell from exiting meiosis I. Because Cdc14 is required for condensin reloading, our data support the idea that condensin functions downstream of Cdc14 in rDNA segregation. Removal of Spo11 activity can alleviate rDNA segregation defects in both condensin and *cdc14* mutants, further indicating that these two rDNA segregation mechanisms are interconnected. We speculate that Cdc14-mediated reloading of condensin onto the rDNA is necessary for DSB repair and hence proper rDNA segregation during yeast meiosis.

In conclusion, we showed that condensin is enriched at the repetitive rDNA gene array to suppress DSB formation and to promote DSB processing during yeast meiosis and thus allow the proper segregation of rDNA homologues at anaphase I. Genomes of higher eukaryotes are rich in repeated DNA sequences; our work may provide insight into the function of condensin in these organisms.

MATERIALS AND METHODS

Yeast strains and culture method

Yeast strains used in our study were diploid derivatives of SK1 (Supplemental Table S1). Condensin mutant alleles, including P_{CLB2}-BRN1, ycs4-2, and ycg1-2, have been described previously (Yu and Koshland, 2003; Brito et al., 2010). We used a PCR-based approach (Longtine et al., 1998) to generate the P_{CLB2}-AID-RPA43 allele, which is conditional during meiosis, and the Rpa43 protein is subject to
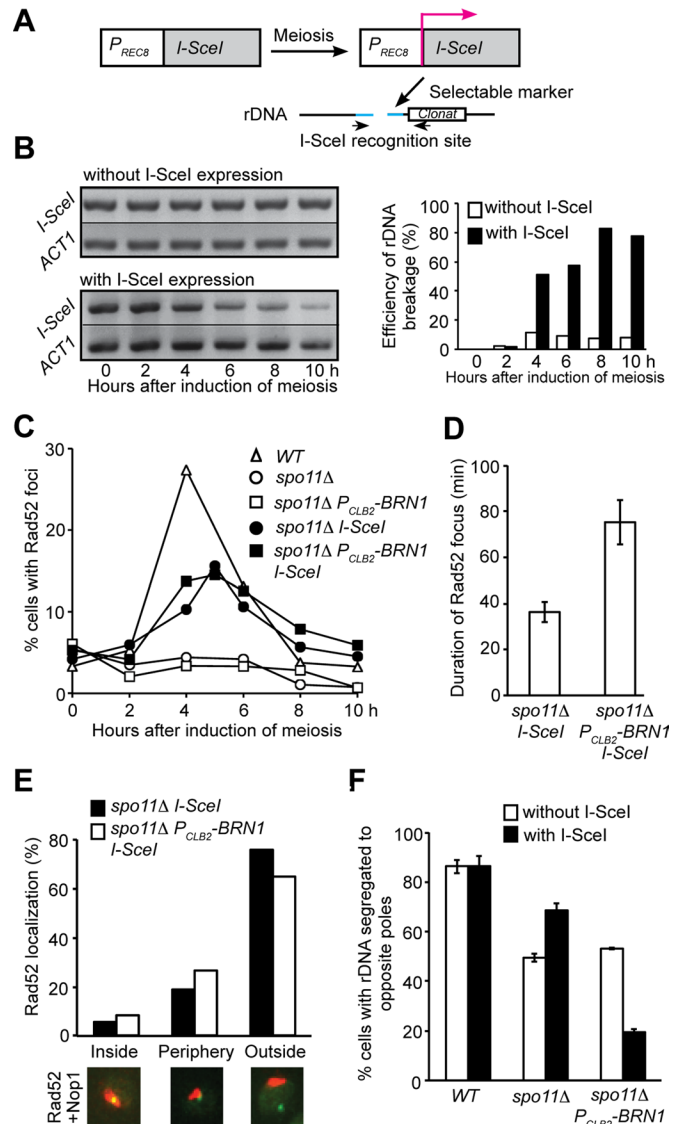

FIGURE 7: Requirement of condensin for repair of an artificial rDNA break during meiosis. (A) Schematic diagram showing the generation of an inducible DSB at the rDNA. (B) PCR-based assay of chromosome breakage at the rDNA (HY3050 and HY 4490). I-SceI fragment was amplified with one primer from the rDNA and another from the selectable marker *Clonat,* as indicated in A. The control fragment was from *ACT1*. Right, quantification of break formation at the rDNA. (C) Rad52 focus formation in yeast cells with an inducible rDNA break during meiosis. Yeast strains (HY3831, HY3887, HY3899, HY3900, and HY3901) were induced to undergo synchronous meiosis; Rad52 focus formation was determined by fluorescence microscopy. (D) Duration of Rad52 focus in meiotic yeast cells with an induced rDNA break (HY3831 and HY3887). (E) Localization of Rad52 foci in live meiotic cells. Three classes of Rad52-GFP (green) localization were found: inside the nucleolus (Nop1-RFP, red), at the periphery of the nucleolus, and outside of the nucleolus. (F) Segregation of rDNA in the presence of an inducible DSB. Yeast strains HY3050, HY3456, HY3458, HY4488, HY4489, and HY4490 all harbored the I-SceI recognition sequence, but only HY3050, HY3456, and HY3458 produced I-SceI.

degradation upon the addition of IAA (Nishimura et al., 2009). A similar PCR-based approach (Longtine et al., 1998) was used to tag the C-termini of Brn1, Ycg1, Cdc14, Tub1, Tub4, and Hta1 with 3x-hemagglutinin (3HA), 6Myc, GFP, or RFP. Primers are available upon request.
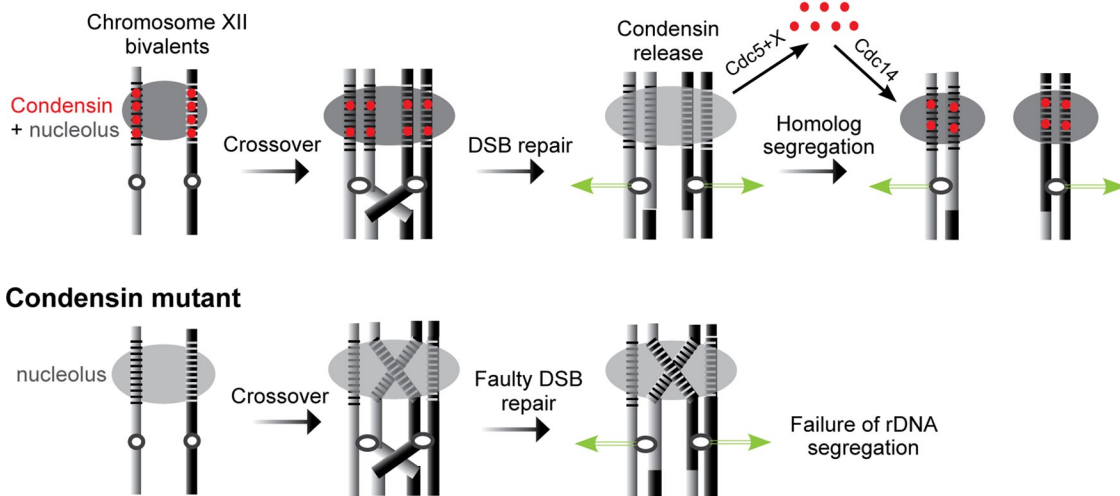
Wild type

Condensin mutant

FIGURE 8: Model for condensin action at the rDNA in yeast meiosis. For simplicity, only rDNA-associated condensins (red dots) are shown. Chromosomes are shown as gray and black bars. In wild-type cells, condensin is enriched at the rDNA at prophase I, resulting in little DSB formation at rDNA. Condensin is released from the rDNA at the prophase I/metaphase I transition and then is reloaded at anaphase I during chromosome segregation. Release of rDNA-associated condensin is regulated by Cdc5 and other unknown factors, whereas reloading of condensin at anaphase I requires Cdc14. In the absence of condensin function, rDNA breaks are formed but not properly repaired, leading to unresolvable homologue linkage.

To visualize the rDNA array in live yeast cells by fluorescence microscopy, we incorporated an array of 5xtetO into each rDNA repeat. TetR-GFP or tetR-RFP was transformed into the rDNA-tetO strains to illuminate the entire rDNA region as reported previously (Li *et al.*, 2011). To mark chromosome XII at its centromere and right telomere, we inserted an array of 224 copies of tetO at the foregoing corresponding loci by homologous recombination (Machin *et al.*, 2005). Similarly, we inserted the tetO array at *TRP1*, *LYS4*, and the right telomere of chromosome IV to mark centromere IV, the right arm of chromosome IV, and its right telomere using plasmids pHG179, 180, and 181, respectively.

To ectopically express *CDC5* in yeast meiosis, we constructed plasmid pHG292, which carries the *CDC5* open reading frame under the control of the *CUP1* promoter, as we described previously (Shirk *et al.*, 2011). We used 50 µM CuSO₄ to induce the expression of *PCUP1-CDC5* at 4 h after induction of meiosis.

For determining the execution point of Ycg1 during meiosis, we used a *GAL4.ER P_GAL-NDT80* allele (Carlile and Amon, 2008) to arrest *ycg1-2* cells at pachytene due to the lack of Ndt80 (Figure 6). Yeast cells were grown in yeast extract, peptone, potassium acetate (YEPA) medium overnight at 25°C, then split into two cultures with equal volumes and induced to undergo meiosis at either 25 or 34°C for 6 h (Yu and Koshland, 2003). The foregoing yeast cultures were then shifted to either 34 or 25°C upon the addition of 1 µM estradiol (final concentration), which released yeast cells from pachytene arrest (Figure 6A).

To introduce an artificial DSB at the rDNA, we inserted one copy of the I-*Sce*I recognition sequence (Galli and Schiestl, 1998) at the 25S rRNA gene by a PCR-based approach. Primer sequence information is available upon request. The copy number of the I-*Sce*I sequence was confirmed by quantitative PCR. Expression of I-*Sce*I was under the control of the meiosis-specific *REC8* promoter (Lin *et al.*, 2011), using the plasmid pHG163.

Unless otherwise stated, yeast cultures were induced to undergo synchronous meiosis at 30°C as we described previously (Yu and Koshland, 2003).

Live-cell fluorescence microscopy

Yeast live-cell microscopy was carried out on a DeltaVision imaging system (Applied Precision, Issaquah, WA) as described previously (Li *et al.*, 2011). We induced yeast cells to undergo meiosis in the sporulation medium, prepared slides, and then performed time-lapse live-cell fluorescence microscopy. For temperature-sensitive yeast mutants and their wild-type controls, live-cell images were acquired at either 25 or 34°C with 3-min time lapses. Optical sections were set at 0.5-µm thickness, and ~14 z-sections were acquired for each time point. To determine condensin dynamics, live-cell images of the cells with Brn1-GFP, or Ycg1-GFP, and rDNA-RFP were acquired with 10-min time lapses. To minimize photobleaching and phototoxicity, optical sections were set at 1-µm thickness with seven z-sections. Acquired images were deconvolved with SoftWorx, and projections were used for display.

Immunofluorescence microscopy

Yeast nucleus surface spreads and immunofluorescence were performed as described previously, with a slight modification (Yu and Koshland, 2003). For yeast strains carrying the *GAL4.ER P_GAL-NDT80 ycg1-2* alleles, cells were spheroplasted at either 25 or 34°C. An anti-HA antibody (12CA5; Roche, Indianapolis, IN) was used to detect HA-epitope–tagged proteins (0.5 µg/ml). An anti-GFP monoclonal antibody (1:2000 dilution, JL-8; Clontech, Mountain View, CA) was used to localize rDNA-GFP. An anti-Rad51 antibody (1:2000 dilution; provided by D. Bishop, University of Chicago, Chicago, IL) was used to detect Rad51 foci. Fluorescence images were acquired with a Plan Apochromat 100×/1.40 numerical aperture objective lens mounted on an AxioImager microscope (Carl Zeiss, Oberkochen, Germany).

Gene conversion assay

We incorporated a *URA3* marker gene at the 25S rDNA gene by transforming yeast cells with a PCR product. Primer sequence information is available upon request. Yeast temperature-sensitive mutants were induced to undergo meiosis at a semipermissive

temperature (30°C). Tetrads were dissected, and spores were germinated at 25°C; tetrads with four viable spores were then genotyped (Figure 2A).

Two-dimensional gel mapping of recombination intermediates

A previously described 2D mapping method was modified to detect Holliday junction molecules at the recombination hot spot *NTS1* region of the rDNA (Brewer and Fangman, 1988). Briefly, yeast cells were harvested from synchronous meiotic cultures 4 h after induction of meiosis. Yeast DNA was extracted and purified by a CsCl method (Brewer and Fangman, 1988). First-dimension gels were run at 1 V/cm and second-dimension gels at 6 V/cm. A standard Southern blot protocol was followed. The intensity of the labeled probes was determined with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). ImageQuant software was used for the quantification of signal intensity.

Immunoblotting

Yeast protein extraction and immunoblot were performed as previously described (Yu and Koshland, 2003). Yeast cells were induced to undergo synchronous meiosis, and aliquots were withdrawn at 2-h intervals. Yeast total proteins were separated by SDS–PAGE, followed by immunoblotting with an ECL kit (Pierce, Rockford, IL).

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