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Rec8 Guides Canonical Spo11 Distribution along Yeast Meiotic Chromosomes

Kazuto Kugou,*†‡ Tomoyuki Fukuda,*† Shintaro Yamada,* Masaru Ito,* Hiroyuki Sasanuma,*† Saori Mori,§ Yuki Katou,§ Takehiko Itoh,‖ Kouji Matsumoto,‡ Takehiko Shibata,† Katsuhiko Shirahige,§ and Kunihiro Ohta*†

*Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Tokyo 153-8902, Japan; †Cellular and Molecular Biology Laboratory, RIKEN Advanced Science Institute, Wako, Saitama 351-0198, Japan; ‡The Graduate School of Science and Engineering, Saitama University, Saitama 338-8570, Japan; §Center for Biological Resources and Informatics, Division of Gene Research, and Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Kanagawa 226-8501, Japan; and ‖Laboratory of Genome Informatics, Bio-Frontier Research Center, Tokyo Institute of Technology, Yokohama, Kanagawa 226-8501, Japan

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Spo11-mediated DNA double-strand breaks (DSBs) that initiate meiotic recombination are temporally and spatially controlled. The meiotic cohesin Rec8 has been implicated in regulating DSB formation, but little is known about the features of their interplay. To elucidate this point, we investigated the genome-wide localization of Spo11 in budding yeast during early meiosis by chromatin immunoprecipitation using high-density tiling arrays. We found that Spo11 is dynamically localized to meiotic chromosomes. Spo11 initially accumulated around centromeres and thereafter localized to arm regions as premeiotic S phase proceeded. During this stage, a substantial proportion of Spo11 bound to Rec8 binding sites. Eventually, some of Spo11 further bound to both DSB and Rec8 sites. We also showed that such a change in a distribution of Spo11 is affected by hydroxyurea treatment. Interestingly, deletion of *REC8* influences the localization of Spo11 to centromeres and in some of the intervals of the chromosomal arms. Thus, we observed a lack of DSB formation in a region-specific manner. These observations suggest that Rec8 would prearrange the distribution of Spo11 along chromosomes and will provide clues to understanding temporal and spatial regulation of DSB formation.

INTRODUCTION

Meiotic recombination is an essential process for viability and acquisition of genetic diversity of gametes during sexual reproduction. It is initiated by programmed DNA double-strand breaks (DSBs), which are transiently introduced at recombination initiation sites after the completion of premeiotic DNA replication (Gerton and Hawley, 2005; Hochwagen and Amon, 2006).

DSB formation is likely regulated at several levels of chromosomal structures (local chromatin, sister chromatid cohesion, axial element components, homologous interaction, and higher order chromosome structures). For example, chromatin remodeling factors and histone modifications such as acetylation, methylation, and ubiquitination are involved in the meiotic alteration of the local chromatin structure at DSB sites (Sollier *et al*., 2004; Yamada *et al*., 2004; Yamashita *et al*., 2004; Mieczkowski *et al*., 2007). In addition, genome-wide studies have shown that DSB regions are distributed nonrandomly in DSB hot and cold domains (Baudat and Nicolas, 1997; Gerton *et al*., 2000; Borde *et al*., 2004; Blitzblau *et al*., 2007; Buhler *et al*., 2007) and that DSBs are formed preferentially in regions of chromatin loops (Blat *et al*., 2002).

Meiotic DSBs are formed by the conserved topoisomerase-like enzyme Spo11, which works with some other proteins (Keeney, 2001). However, a regional difference in DSB competency does not simply reflect the regulation of Spo11 binding to the chromosomes, because the targeting of Spo11 fused with Gal4 DNA binding domain (Gal4BD-Spo11) to cold domains such as yeast centromeric regions cannot induce DNA cleavage during meiosis (Robine *et al*., 2007; Fukuda *et al*., 2008). To uncover the regional regulation of Spo11, it is necessary to determine the chromosome-wide dynamic distribution of Spo11 in wild-type cells. Previous genome-wide studies of the Spo11 distribution along yeast chromosomes were performed in the *rad50S*-like mutant background, in which DSB ends are left unprocessed with covalently bound Spo11 (Alani *et al*., 1990), providing the map that does not taken into account the dynamic features of the process (Baudat and Nicolas, 1997; Mieczkowski *et al*., 2006, 2007; Robine *et al*., 2007). The binding sites of Spo11 in *rad50S*-like mutants have been considered as DSB formation

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Address correspondence to: Kunihiro Ohta (kohta@bio.c.u-tokyo.ac.jp).

Abbreviations used: ChIP, chromatin immunoprecipitation; DSB, double-strand break; Gal4BD, Gal4 DNA binding domain; HU, hydroxyurea; SPM, sporulation medium.

sites. Recently, DSB sties were genome-widely mapped by detecting single-strand DNA sites in the deletion mutant of *DMC1*, a eukaryotic RecA homologue, which catalyzes the single-strand invasion (Blitzblau *et al*., 2007; Buhler *et al*., 2007). In the mutant, Spo11 is removed from the DSB ends, but unrepaired single-strand DNA accumulates (Bishop *et al*., 1992). Similar distribution of DSB was observed in both types of mutants except for some regions: in the *dmc1*Δ mutant, DSBs were formed also in the DSB cold domains located near centromeres and chromosome ends in the *rad50S* like mutant (Blitzblau *et al*., 2007; Buhler *et al*., 2007).

Another important aspect of the regional control of Spo11-mediated DSB formation is sister chromatid cohesion. In meiosis, a specific component of cohesin, Rec8, plays important roles in meiotic chromosomal metabolism (Stoop-Myer and Amon, 1999). In *Schizosaccharomyces pombe*, Rec8 mutant exhibits loss of proper segregation of homologous chromosomes, linear element formation, and mono-oriented kinetochores (Krawchuk *et al*., 1999; Watanabe and Nurse, 1999). In addition, *S. pombe rec8*Δ mutant shows a marked reduction of meiosis-specific DNA breakage by Rec12 (the *S. pombe* Spo11 homologue) in several intervals of the genome, but less in others (Ellermeier and Smith, 2005). However, in *Saccharomyces cerevisiae*, the deletion of *REC8* has reportedly no effect on meiotic DSB formation when observed at a couple of DSB sites on chromosome III (Klein *et al*., 1999). Thus, universal roles of Rec8 in the regulation of DSB formation have not been fully elucidated yet.

In this study, we examined the distribution of Spo11 along meiotic chromosomes in wild-type budding yeast cells, and we compared it with that of Rec8, by using a high-resolution genome tiling array and chromatin immunoprecipitation (ChIP) assay (Katou *et al*., 2003; Lengronne *et al*., 2004). We demonstrate that Spo11 is first recruited to centromeric regions, and then relocalizes to arm regions, in concert with the progression of the premeiotic DNA replication. Initial centromeric entry of Spo11 depends upon Rec8. In the *rec8*Δ mutant, Spo11 no longer binds to centromeres in early meiotic prophase. In addition, in the *rec8*Δ mutant, meiotic DSBs formation and the binding of Spo11 to DSB sites are severely impaired at selective domains of many chromosomes other than the previously studied chromosome III. These results suggest that Rec8 at centromeres and cohesion sites choreographs the distributions of Spo11 to DSB sites during premeiotic DNA replication.

MATERIALS AND METHODS

Yeast Strains and Plasmid

All strains were SK1 background and are listed in Supplemental Table S1. 6His-3FLAG, 6His-3HA, or 6His-2myc tag was fused to the C terminus of the protein by using a cassette amplified from pU6H3FLAG, pU6H3HA, or pU6H2myc (De Antoni and Gallwitz, 2000; Katou *et al*., 2003). The *rec8* null allele was introduced by polymerase chain reaction (PCR)-mediated gene disruption. All strains with the *rad50S* allele were obtained by crossing and tetrad dissection. All strains and plasmids used in this study are available upon request.

Cultures

For the preparation of meiotic culture, yeast cells were cultured in supplemented presporulation medium and induced to meiosis in sporulation medium (SPM) at 30°C by using a protocol described previously (Ohta *et al*., 1998; Kugou *et al*., 2007). For the preparation of hydroxyurea (HU)-arrested cells, HU stock solution (1 M HU dissolved in SPM) was added to a final concentration of 100 mM at 15 min after meiotic induction. The progression of meiosis was monitored by fluorescence-activated cell sorting (FACS) analysis and nuclear staining with Hoechst 33342.

ChIP Analyses and Quantitative Real-Time PCR

ChIP was performed as described previously (Katou *et al*., 2003), with some modifications. Nontagged strains were treated as a negative control. In brief, cells were fixed with 1% formaldehyde for 10 min and then treated with 125 mM glycine for 5 min at room temperature. Cells were suspended in 140 mM NaCl-containing lysis buffer with Complete proteinase inhibitor (Roche Diagnostics, Mannheim, Germany) and then disrupted by the Multi-Beads Shocker (Yasui Kikai, Osaka, Japan). Immunoprecipitation of 3×FLAG, 3×hemagglutinin (HA), or 2× myc-tagged protein was performed with anti-FLAG antibody M2 (Sigma-Aldrich, St. Louis, MO), anti-HA antibody ab9110 (Abcam, Cambridge, United Kingdom) or anti-myc antibody 4A6 (Millipore, Billerica, MA), respectively. The DNA was analyzed by real-time PCR 7300 system (Applied Biosystems, Foster City, CA) with SYBR premix *Ex Taq* (Takara Bio USA, Madison, WI). The primer pairs listed in Supplemental Table S2 were used. Three independent PCR reactions were performed to calculate the means, and the results are indicated as ratios of immunoprecipitated DNA versus input DNA.

ChIP-Chip Analyses

Preparation of immunoprecipitated DNA and input DNA was performed as described above. For mapping of DSB sites in *rad50S* mutants, cells at 7 h after meiotic induction were treated as described above but without formaldehyde treatment. The immunoprecipitated DNA and input DNA were amplified by random priming, fragmented, end labeled, and hybridized to two types of high-density oligonucleotide array (SC3456a520015F, P/N 520015 and rikDACF, P/N 510636; Affymetrix, Santa Clara, CA) as described previously (Katou *et al*., 2003; Lengronne *et al*., 2004). These two chips cover the entire yeast chromosome VI (rikDACF), or chromosome III, IV, V, and the right arm of VI (SC3456a520015F), with a resolution of 300 base pairs and 100 base pairs (within 17-kb around *YCR048W*, chromosome III 198101 base pairs and 215100 base pairs, on SC3456a520015F), except for some repeat sequences (Ty LTR, telomeric region, rRNA and some other genes, indicated with white boxes in Supplemental Figure S1). To draw actual binding sites, the primary data set obtained by Affymetrix GeneChip system (Hp GeneArray Scanner and Microarray Suite 5 or GeneChip Scanner 3000 7G and GeneChip operating software 1.4) was further analyzed based on the p value criteria described previously (Katou *et al*., 2003). The microarray data presented in this article have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo) and are accessible through GEO series accession GSE8422.

DSB Detection by Southern Blotting

DNA samples were prepared from meiotic *rad50S* cells as described previously (Borde *et al*., 1999). The blots were hybridized with randomly ^{32}P-labeled probes of PCR fragments amplified with the primer pairs listed in Supplemental Table S2 by using yeast genomic DNA as template.

RESULTS

Bindings of Spo11 before Premeiotic S Phase

To study interaction of Spo11 and Rec8 with chromosomes by ChIP-chip, we fused a FLAG-epitope tag at the C terminus of Spo11 and Rec8 (referred to as Spo11-FLAG and Rec8-FLAG) and expressed them in yeast cells under their original promoters. We confirmed that these strains do not exhibit any defects in their meiotic functions (i.e., progression of premeiotic S phase, meiotic recombination, meiotic division, spore formation, and spore viability; Supplemental Table S3 and Supplemental Figure S2). In addition, we validated our ChIP procedure by observing that Spo11-FLAG binds to the *YCR048W* DSB hot spot, but little to the *CWH43* DSB cold spot during meiosis (Supplemental Figure S3).

We examined chromosome-wide distributions of Spo11-FLAG and Rec8-FLAG in "*RAD50*$^{+}$ wild-type" cells by using a genome tiling array (Katou *et al*., 2003; Lengronne *et al*., 2004) that covers the chromosome III, IV, V, and VI (see *Materials and Methods*). Bindings of Spo11-FLAG to chromosomes during premeiotic culture (i.e., time 0) could not be observed (data not shown). At the 1.5 h after meiotic induction, when premeiotic DNA replication is about to start (see Figure 1A and Supplemental Figure S4), initial binding of Spo11-FLAG on chromosome VI was detected mainly within the 20- to 30-kb regions harboring the centromere (referred to as pericentromeric regions in this study) and at some scattered sites on the chromosome arms (Figure 1B). The ChIP-chip experiments for Rec8-FLAG demonstrated that Rec8 also initially bound pericentromeric regions at the

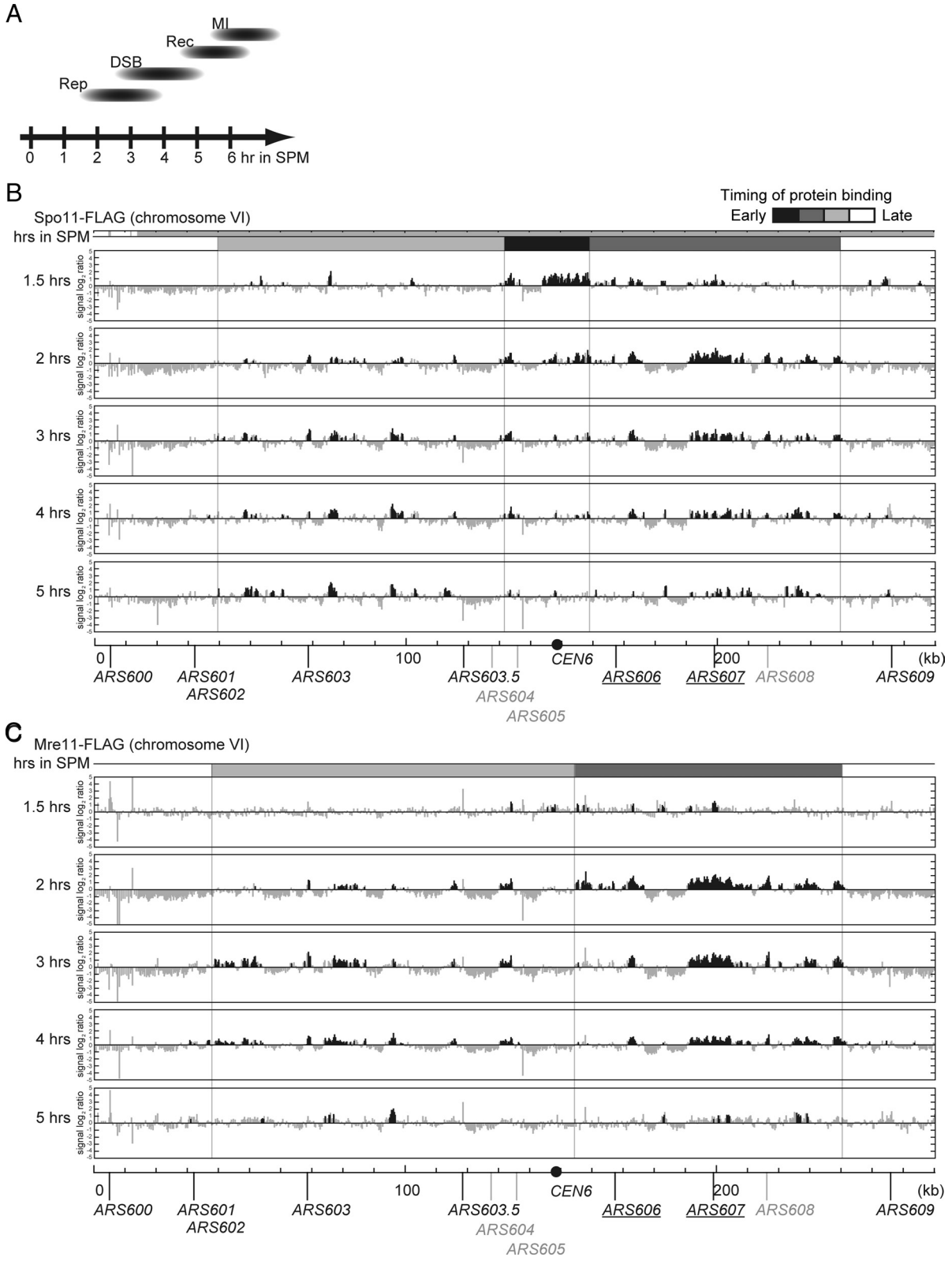

Figure 1. Distributions of Spo11-FLAG and Mre11-FLAG on meiotic chromosome VI. (A) Summary of meiotic events in SK1 background strain. Rep, premeiotic DNA replication; DSB, DSB formation; Rec, homologous recombination; MI, meiosis I. (B and C) ChIP-chip analyses of Spo11-FLAG (B) and Mre11-FLAG (C). RKD1311 (*SPO11-FLAG*) and RKD1313 (*MRE11-FLAG*) cells were cross-linked at indicated time points after the medium change and analyzed. The horizontal axis represents the physical position on the chromosome. The vertical axis represents the relative binding strength of these proteins as compared with the Input signals. The scale of the vertical axis is expressed in $\log_2$. Black and light gray vertical bars represent detection loci showing significant binding ratios and insufficient enrichment in the immunoprecipitated fraction, respectively. The position of the centromere is indicated with the black circle on the horizontal axis. Timing of the

1.5 h after meiotic induction (Figure 2A). Similar features also were observed on chromosomes III–V at the same time (Figure 2A), suggesting that the pericentromeric regions may function as the initial binding sites of Spo11 and Rec8.

DSBs formation has not been detected at inside centromeres, and it is likely to be reduced in 8- to 10-kb regions from centromeres in *S. cerevisiae* (Blitzblau *et al*., 2007; Buhler *et al*., 2007). To validate the binding of Spo11-FLAG to the pericentromeric regions, we performed quantitative PCR-based ChIP experiments (Figure 2, B–D). Spo11-FLAG bound to the *CEN3* at 1.5 h after meiotic induction before the binding to the *YCR048W* DSB hot spot and stayed until 4 h after meiotic induction (Figure 2C). The binding signals to *CEN3* were also obtained with HA- or myc-tagged Spo11 (Figure 2E).

We next examined whether Mre11, which is a component of DSB formation complex and needs Spo11 protein to associate with DSB hot spot (Borde *et al*., 2004), binds to the pericentromeric regions during early meiosis. We fused a FLAG tag to the C terminus of Mre11, checked its meiotic functions, and performed ChIP experiments and ChIP-chip experiments (Supplemental Table S3 and Supplemental Figure S3). In contrast to Spo11-FLAG, Mre11-FLAG did not show remarkable binding to the pericentromeric regions at 1.5–5 h after meiotic induction and seemed to be distributed randomly along chromosome arms at 1.5 h after meiotic induction (Figures 1C and 2, A and D). These results indicate that Spo11 might have unknown functions, rather than DSB formation itself, at the pericentromeric region, as proposed for the *S. pombe* Spo11 homologue Rec12 (Ludin *et al*., 2008).

Localization of Spo11 to Chromosomal Arms during Premeiotic S Phase

In our experimental conditions, premeiotic DNA synthesis occurred mainly at 2–3 h after meiotic induction (cf. Figure 1A and Supplemental Figure S4). At 2 h of meiosis, both Spo11-FLAG and Mre11-FLAG began to be localized in concert to an early replicating region of chromosome VI (Friedman *et al*., 1997; Yamashita *et al*., 1997; Raghuraman *et al*., 2001; Mori and Shirahige, 2007), including the very early mitotic and meiotic replication origins autonomously replicating sequences (*ARS*)*606* and *ARS607* (Figure 1, B and C). As premeiotic DNA replication further proceeded, they gradually localized to the chromosomal arm regions harboring comparatively late-replicating origins (Pearson *r* of signal $\log_2$ ratio between Spo11-FLAG and Mre11-FLAG is 0.85 at 2 h and 0.72 at 3 h; Figure 1, B and C). Similar results were observed on chromosome III, IV, and V (cf. Supplemental Figure S5).

Rec8-FLAG was localized to discrete sites on chromosomal arms during premeiotic S phase (Figure 3). Remarkably, a substantial portion of Spo11-FLAG was bound around the binding sites of Rec8-FLAG (Figure 4, A–C, and Tables 1–3), indicating that Spo11 and Rec8 may be localized to common binding sites on arm regions.

Figure 1 (cont). Spo11-FLAG and Mre11-FLAG binding along chromosome VI is indicated by shaded boxes (from black to white) above the top panel. Shaded parts under the coordinate axis in B represent detection loci for the mapping (for details, see Supplemental Figure S1). Names and locations of known *ARS*s are shown at the bottom of each figure. Underlined *ARS* and light gray-colored *ARS* represent active early and inactive replication origins, respectively.

Localization of Spo11 to Rec8 Binding Sites and DSB Hot Spots

As reported previously (Blitzblau *et al*., 2007; Buhler *et al*., 2007), DSB sites detected in the *rad50S* mutant (i.e., noncross-linked Spo11-FLAG binding sites in *rad50S*) are generally similar to but partly different from those in the *dmc1*Δ mutant (i.e., single-stranded DNA-enriched regions in *dmc1*Δ) (see Figure 4D). Many of the Spo11-FLAG binding sites seemingly localized around the DSB sites observed in the *rad50S* mutant from 3 to 4 h of meiosis. However, some of Spo11-FLAG also bound to the DSB sites detected only in the *dmc1*Δ mutants (Figure 4D), especially later time points (e.g., 4–5 h). Interestingly, colocalization of Spo11-FLAG and Rec8-FLAG decreased from 4 to 5 h of meiosis (Figure 4, A–C, and Tables 1–3).

Most of the Rec8 remained at the initial positions until the onset of meiosis I, except for a few where Rec8 position was slightly shifted, possibly mediated by local transcriptional activation of meiosis-specific genes (e.g., *PES4*; Figure 3, black arrow; Lengronne *et al*., 2004). The Rec8 sites did not colocalize with the DSB sites detected in *rad50S* mutant and *dmc1*Δ mutant (Table 3), as reported in previous studies (Glynn *et al*., 2004; Cromie *et al*., 2007).

Effect of DNA Replication Block on the Binding of Spo11 to Chromosome Arms

Spo11-FLAG bound to arm regions at a similar timing with the premeiotic DNA replication (Figure 1B and Supplemental Figure S5). This led us to consider the possibility that the binding of Spo11-FLAG to the chromosome arms may be coupled to DNA replication-related processes. To test this hypothesis, we examined effects of HU treatment, which blocks DNA replication, on the chromosomal distribution of Spo11-FLAG by ChIP-chip analyses. Addition of HU completely inhibited premeiotic DNA replication and drastically reduced meiotic recombination at the *arg4-bgl/arg4-nsp* heteroallele (Figure 5, A–C).

At 2 h after adding HU, Spo11-FLAG proteins could localize on the initial binding sites of the normal condition, i.e., the Spo11-FLAG binding sites on the chromosome VI at 1.5 h of meiosis without HU (half of its binding sites overlapped with the initial binding sites; Figure 5D).

After longer HU treatment (4 h), in addition to the binding of Spo11-FLAG to the initial binding sites (~50% of them still remained), Spo11-FLAG exhibited rather broader distributions around early replicating origins (Figure 5D). Notably, binding of Spo11-FLAG to DSB hot spots was detected in the early replicating region, but not in the other regions even after 4 h in meiotic culture (e.g., *YFR038W* DSB hot spot; filled arrowheads in Figure 5D). Similar results were obtained on chromosome III (cf. Supplemental Figure S6). These results suggest that a replication block may affect the distribution of Spo11 to some of DSB sites.

Effects of REC8 Deletion on the Distribution of Spo11 and DSBs

The correlation between the Spo11-FLAG and the Rec8-FLAG binding sites in the early stages of meiosis led us to examine whether the deletion of *REC8* affects the distribution of Spo11. Intriguingly, in the *rec8*Δ mutant, Spo11-FLAG could hardly localize to the pericentromeric region throughout the early time points (1.5–4 h) on all of the chromosomes examined (Figure 6 and Supplemental Figure S7, indicated with arrows). It is also noteworthy that *rec8*Δ conferred a perturbation of the Spo11-FLAG distribution on the chromosome arms in a region-dependent manner. In

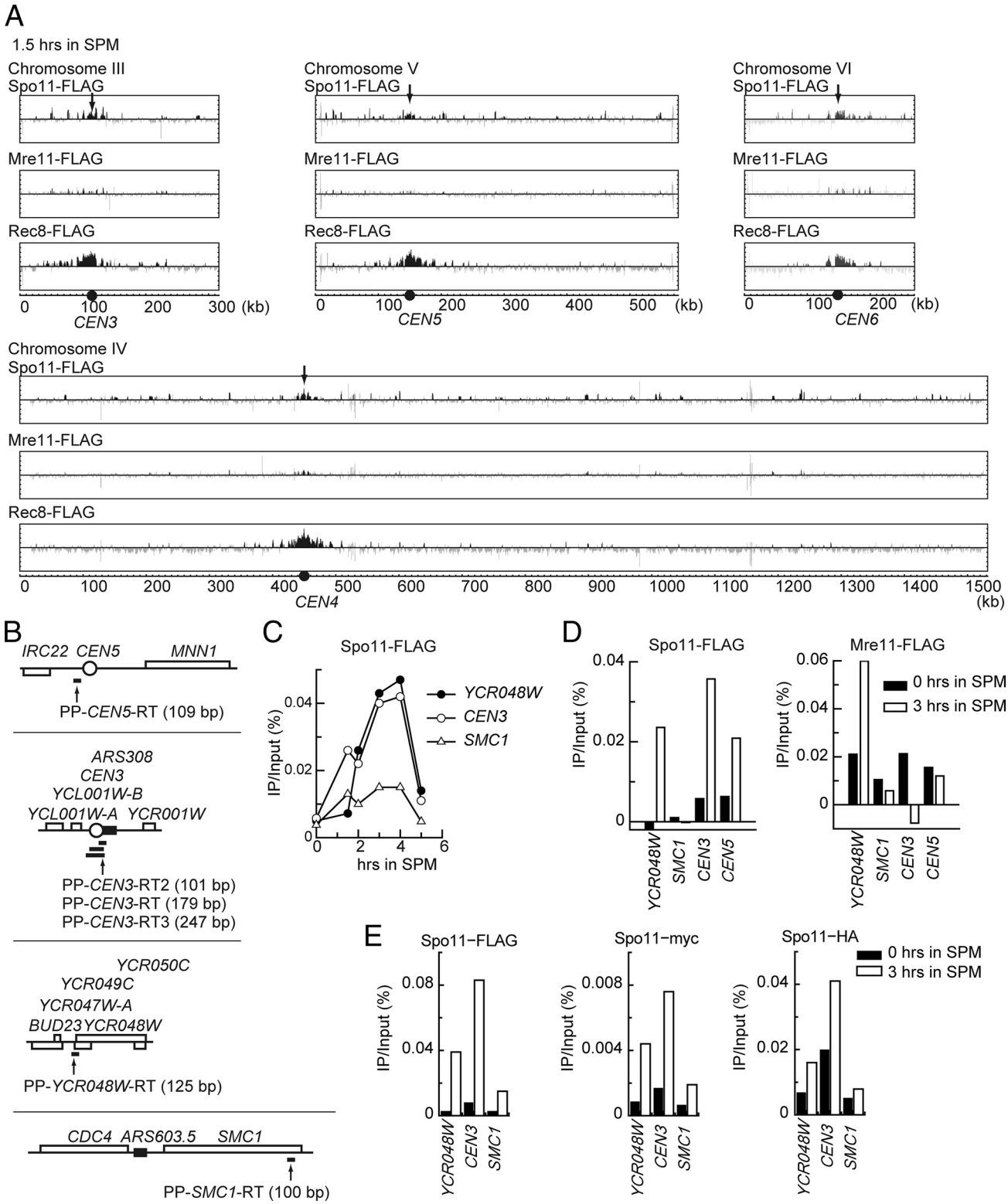

Figure 2. Spo11-FLAG localized to the centromeres at early meiosis. (A) Localization of Spo11-FLAG (RKD1311), Mre11-FLAG (RKD1313), and Rec8-FLAG (RKD1317) at 1.5 h after transfer to SPM on chromosome III–VI. All indications are as described in Figure 1. (B) Map of the primer pairs (PP) used for quantitative real-time PCR (qPCR). Open circle, open boxes, closed boxes, and horizontal thick bars indicate centromere, open reading frames, *ARS*s and PCR fragments, respectively. (C) ChIP-qPCR of Spo11-FLAG. The Spo11-FLAG binding to *CEN3* was analyzed by qPCR using PP-*CEN3*-RT. (D) Localization of Spo11-FLAG and Mre11-FLAG at centromeres, a DSB hot spot and a DSB cold spot. The bindings of Spo11-FLAG and Mre11-FLAG to *CEN3* and *CEN5* were detected by qPCR using primer pairs PP-*CEN5*-RT and PP-*CEN3*-RT2, respectively. The ratio (immunoprecipitated/input) was calculated by subtracting the value of no-tagged sample from that of FLAG-tagged sample. (E) Localization of Spo11 to *CEN3* was not affected by different epitope tags. FLAG-tagged (RKD1311), HA-tagged (RKD1329), and myc-tagged Spo11 (RKD1339) were detected on *CEN3* at 3 h in SPM. PP-*CEN3*-RT3 was used for qPCR.

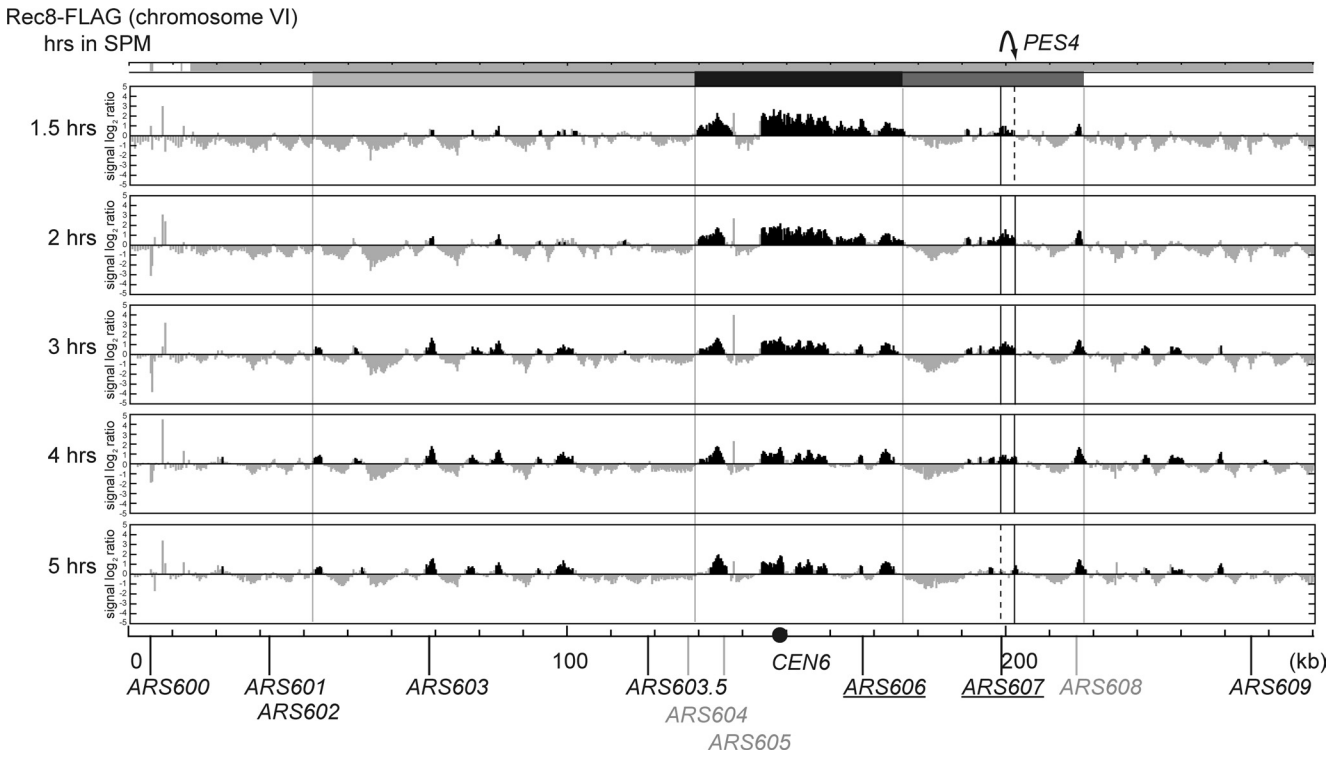

Figure 3. Distributions of Rec8-FLAG on meiotic chromosome VI. All indications are as described in Figure 1. RKD1317 (*REC8*-FLAG) was used in this analysis.

particular, the *REC8* deletion abolished the localization of Spo11-FLAG within the chromosomal domains proximal to the centromeres in the right arm of the chromosome V and VI (Figure 6B and Supplemental Figure S7C), and in both arms of chromosome IV (Supplemental Figure S7, A and B). In contrast, the Spo11-FLAG distribution along the chromosome III was less affected in *rec8*Δ mutant: Spo11-FLAG was localized within ~100-kb regions throughout premeiotic S phase and generally persisted in the same regions, as observed in *REC8*$^+$ cells (1.5 to 4 h, see regions indicated with light gray boxes in Figure 6A).

The region-dependent defect in the Spo11 distribution suggests that the deletion of *REC8* may lead to some region-dependent defect of DSB formation as well, as reported previously in *S. pombe* (Ellermeier and Smith, 2005). Thus, we compared DSB sites in *rad50S rec8*Δ mutant with those in the *rad50S* mutant by ChIP-chip experiments. As predicted, we detected the region-dependent effects of the deletion of *REC8* on DSBs. First, no DSB formations were detected in the domains proximal to the centromeres on chromosome IV, V, and VI in the *rad50S rec8*Δ mutant (Figure 7A and Supplemental Figure S8A, regions indicated with light gray boxes). Second, chromosome III exhibited little effect or even a slight promotion of DSB formation at some sites (Figure 7B, regions indicated with light gray boxes). This observation is consistent with the previous report that *rec8*Δ mutant has little effect on DSB formation at the *YCR048W* hot spot on chromosome III (Klein *et al*., 1999). Third, the deletion of *REC8* in the *rad50S* mutant often exhibited altered DSB frequency in subtelomeric regions. We observed a decrease in DSB formations in subtelomeric regions of chromosome IV and the right arm of chromosome VI, whereas an enhancement of DSB formations was detected in the subtelomeric regions of the left arm of chromosome VI (regions indicated with light gray boxes in Supplemental Figure S8A).

These effects of the *REC8* deletion on DSB formation in the *rad50S* mutant were confirmed by Southern hybridization experiments combined with pulsed-field or conventional gel electrophoreses. Consistent with the present ChIP-chip data (Figure 7B) and the previous Southern blotting analysis (Klein *et al*., 1999), almost normal levels of DSBs were detected in the *rad50S rec8*Δ mutant on the right arm of chromosome III and at some DSB hot spots on other chromosomes, notably within a region around the *YCR048W* (regions indicated with open arrowheads in Figure 7, C and E) and *YDR188W* DSB hot spots (indicated with open arrowheads in Supplemental Figure S8B). More importantly, DSB frequency was reduced very severely on chromosome I, V, and VIII and the left arm of chromosome VII in the *rad50S rec8*Δ mutant, compared with those in the *rad50S REC8*$^+$ cells (Figure 7, D and F, and Supplemental Figure S8C). An enhancement of DSB frequency in the *rad50S rec8*Δ mutant was observed in the subtelomeric regions of chromosome III (Figure 7C, indicated with the filled arrowheads), which is consistent with the ChIP-chip data described above. We estimated that nearly 90% of the DSB sites on chromosome I, V, VIII, and on the left arm of chromosome VII were affected. Similar effects were observed in *dmc1*Δ and *dmc1*Δ *rec8*Δ mutants (Figure 7D). These effects of the *REC8* deletion should not be a consequence of a poor meiotic entry of these mutants, because we observed by FACS analyses that these *rad50S rec8*Δ and *dmc1*Δ *rec8*Δ mutants underwent meiotic entry enough efficiently (Supplemental Figure S4J). Together, we concluded that *S. cerevisiae* Rec8 is involved in the control of distribution of Spo11 and meiotic DSB formations in a region-dependent manner.

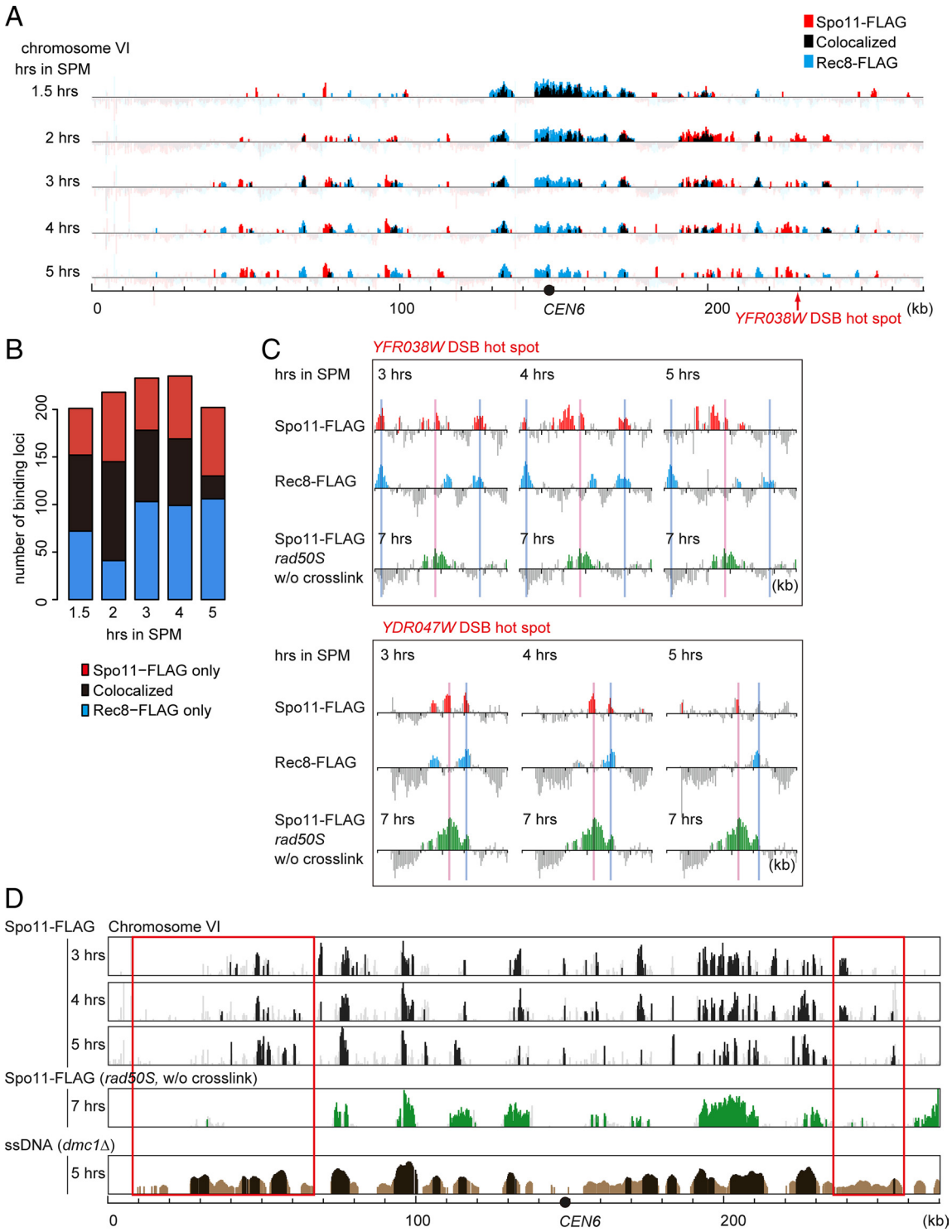

Figure 4. Comparison of DSB sites and binding sites of Spo11-FLAG and Rec8-FLAG. (A) Overlay of Spo11-binding sites (red bars) with Rec8-binding sites (blue bars) on chromosome VI. Overlapped sites are shown as black bars. Other indications are as described in Figure 1. Only detection loci judged as significant binding are shown. (B) Stacked bar graph of numbers of binding loci for Spo11-FLAG only (red), Rec8-FLAG only (blue), and both proteins (black). (C) Magnified views of localization of Spo11-FLAG, Rec8-FLAG, and DSBs (*rad50S*) around *YFR038W* (top) and *YDR047W* DSB (bottom) hot spots during 3–5 h after meiotic induction. The DSB sites were revealed as Spo11-FLAG binding sites by ChIP-chip analysis without cross-linking in RKD1325 (*rad50S SPO11-FLAG*) at 7 h after meiotic induction. Spo11-FLAG binding sites, Rec8-FLAG binding sites, and DSB sites (*rad50S*) are indicated with vertical red, blue, and green bars, respectively. Light red and blue vertical lines represent positions of DSB sites and Rec8-FLAG binding sites, respectively. (D) Comparison of binding sites of Spo11-FLAG in wild-type and DSB sites in *rad50S* (green bars) and *dmc1*Δ (brown bars). Mapping of the DSB sites in *dmc1*Δ was carried out according to Buhler's microarray data (Buhler *et al*., 2007; GEO accession GSE8981). The signal (enrichment of single-stranded DNA) is transformed to $\log_2$. In the lowest panel (single-stranded [ss]DNA *dmc1*Δ), light and dark brown bars represent weak DSB sites (enrichment >2) and strong DSB site (enrichment >5), respectively. DSB cold domains observed only in *rad50S* are indicated with red boxes.

Table 1. Population of Spo11-FLAG binding sites overlapping with DSB sites and Rec8 binding sites on chromosome VI

	Hours in SPM[a]				
	1.5	2	3	4	5
DSB (*dmc1*Δ)[b]	23.3 (30)	42.9 (76)	50.8 (66)	55.1 (75)	55.2 (53)
DSB (*rad50S*)[c]	35.7 (46)	53.7 (95)	53.8 (70)	55.1 (75)	51.0 (49)
Rec8-FLAG[d]	62.0 (80)	58.8 (104)	57.7 (75)	51.5 (70)	25 (24)
Total no. of Spo11-FLAG sites	(129)	(177)	(130)	(136)	(96)

[a] Values are percentages. The number of detection loci determined as binding sites is indicated in parentheses.

[b] Microarray data in Buhler *et al*. (2007) was used in this calculation. Compared with DSB sites, ssDNA enrichment was more than fivefold in *dmc1*Δ at 5 h in meiosis. Two hundred and eighteen detection loci (in our array format) were detected as DSB sites. The overlapping population (percentage) was calculated as (Spo11/DSB-overlapping detection loci)/(total detection loci detected binding sites).

[c] Spo11-FLAG binding sites at 7 h in SPM in *rad50S* determined by ChIP-chip analysis without crosslinking were used in this calculation. One hundred and eighty-eight detection loci were detected as DSB sites. The overlapping population (percentage) was calculated as (Spo11/DSB-overlapping detection loci)/(total detection loci detected binding sites).

[d] The overlapping population (percentage) was calculated as (Spo11/Rec8-overlapping detection loci)/(total detection loci detected as Spo11-FLAG binding sites).

Table 3. Population of Rec8-FLAG binding sites overlapping with DSB sites and Spo11-FLAG binding sites on chromosome VI

	Hours in SPM[a]				
	1.5	2	3	4	5
DSB (*dmc1*Δ)	21.1 (32)	22.8 (33)	21.3 (38)	21.3 (36)	20.8 (27)
DSB (*rad50S*)	36.2 (55)	40.0 (58)	36.0 (64)	34.9 (59)	33.0 (43)
Spo11-FLAG	52.6 (80)	71.7 (104)	42.1 (75)	41.4 (70)	18.5 (24)
Total no. of Rec8-FLAG sites	(152)	(145)	(178)	(169)	(130)

All explanatory materials and footnotes are as described in Table 1.

[a] Values are percentages. The number of detection loci determined as binding site is indicated in parentheses.

DISCUSSION

We demonstrated changes in the distribution of Spo11, Mre11, and Rec8 binding onto meiotic chromosomes by high-resolution genome-tiling arrays combined with ChIP experiments (ChIP-chip; Katou *et al*., 2003; Lengronne *et al*., 2004). The results indicate that Spo11 initially localizes at pericentromeric regions and then distributes to arm regions with progression of premeiotic DNA replication. Substantial portion of Spo11 is colocalized with Rec8 on chromosomal arms at early meiotic-stage (1.5–3 h of meiosis). Thereafter, it also binds to the DSB sites located between the Rec8 sites by the time of full DSB formation (3–5 h). Finally, colocalization between Spo11 and Rec8 is markedly decreased (5 h). Furthermore, the distribution of Spo11 to centromeres and DSB sites is severely impaired in the absence of Rec8 in a region-dependent manner.

Dynamic Distributions of Spo11 along Meiotic Yeast Chromosomes

We found that Spo11 and Rec8 were first concentrated at pericentromeric regions and then localized to relatively early replicating regions. Initial localization of Mre11 on early meiotic chromosomes was less distinct, compared with that of Spo11 and Rec8. However, at later time points, Mre11 binding sites occurred at some discrete sites on the arm regions. After this stage, the Mre11 binding sites were generally superimposed with the Spo11 binding sites. This dif-

Table 2. Summary of chi-square test

Hours in SPM	Observed[a]	Expected[b]	Observed/expected	χ^2	p value
Chi-square test (Spo11-FLAG and Rec8-FLAG)					
1.5	80	27	2.99	159	$<10^{-6}$
2	104	35	2.96	219	$<10^{-6}$
3	75	32	2.37	93.2	$<10^{-6}$
4	70	31	2.23	73.6	$<10^{-6}$
5	24	17	1.40	3.4	0.066
Chi-square test (Spo11-FLAG and DSB (*rad50S*))					
1.5	46	33	1.39	7.5	0.0062
2	95	46	2.09	93.6	$<10^{-6}$
3	70	33	2.10	63.7	$<10^{-6}$
4	75	35	2.14	73.9	$<10^{-6}$
5	49	25	1.98	35.6	$<10^{-6}$
Chi-square test (Spo11-FLAG and DSB (*dmc1*Δ))					
1.5	30	38	0.78	2.9	0.091
2	76	53	1.44	18.4	1.8×10^{-5}
3	66	39	1.70	31.9	$<10^{-6}$
4	75	41	1.85	49.7	$<10^{-6}$
5	53	29	1.85	32.6	$<10^{-6}$

[a] Number of the observed detection loci as overlapping.

[b] Number of the expected detection loci as overlapping.

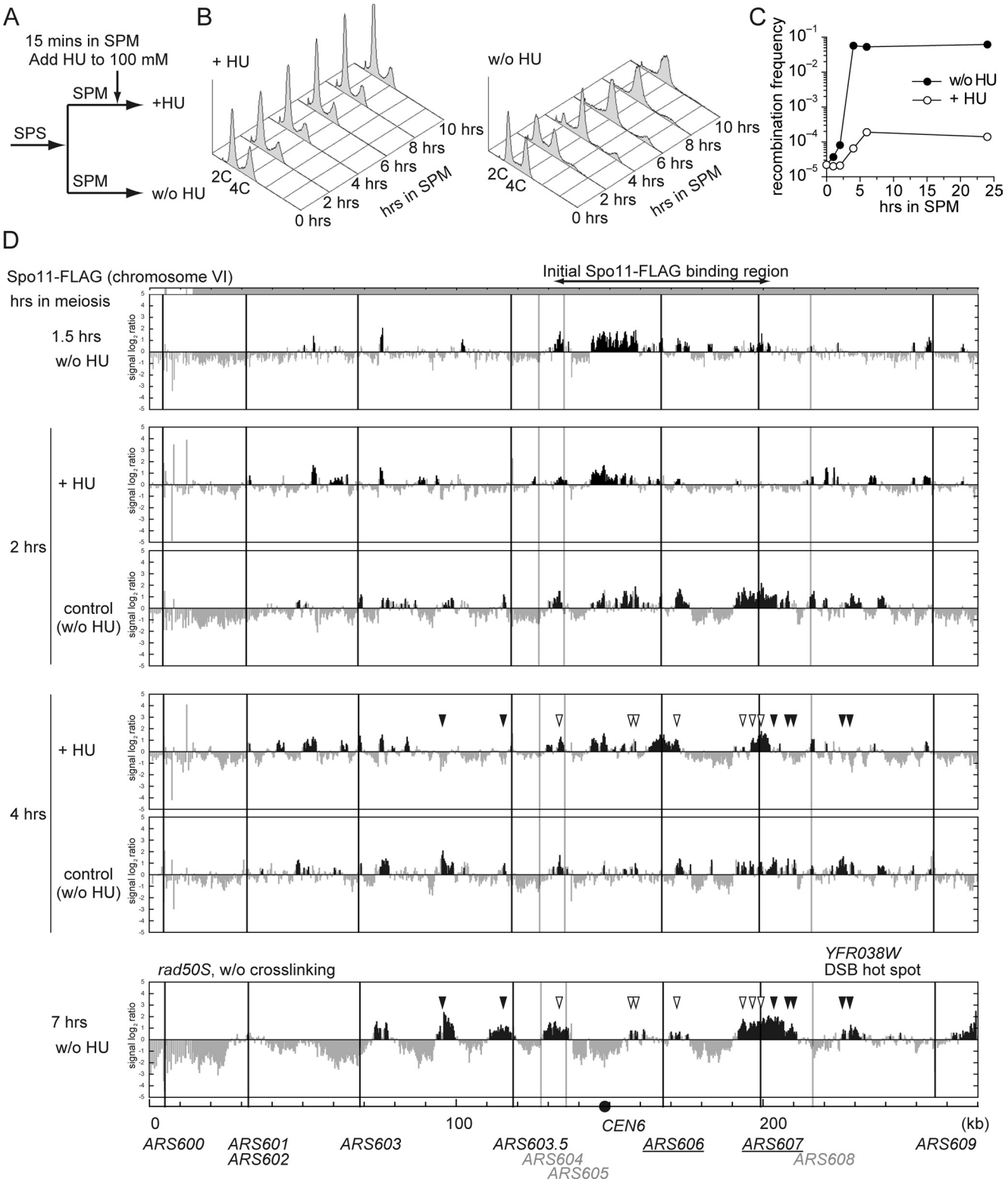

Figure 5. Effects of blocking replication by HU treatment on the distributions of Spo11-FLAG. (A) The culture (RKD1311) was divided into two parts on transferring to SPM. HU was added to one of them 15 min after meiosis induction. (B) Progression of premeiotic S phase monitored by FACS analyses. (C) Recombination frequency measured by return-to-growth at the *arg4-bgl/arg4-nsp* heteroallele. (D) Effects of 100 mM HU treatment on the association of Spo11-FLAG with meiotic chromosome VI. Cells from the same culture as in (B) were taken at 2 and 4 h of meiosis and analyzed. The binding sites of Spo11-FLAG were classified into two subgroups as indicated with filled and open arrowheads (see text). Other indications are as described in Figure 1.

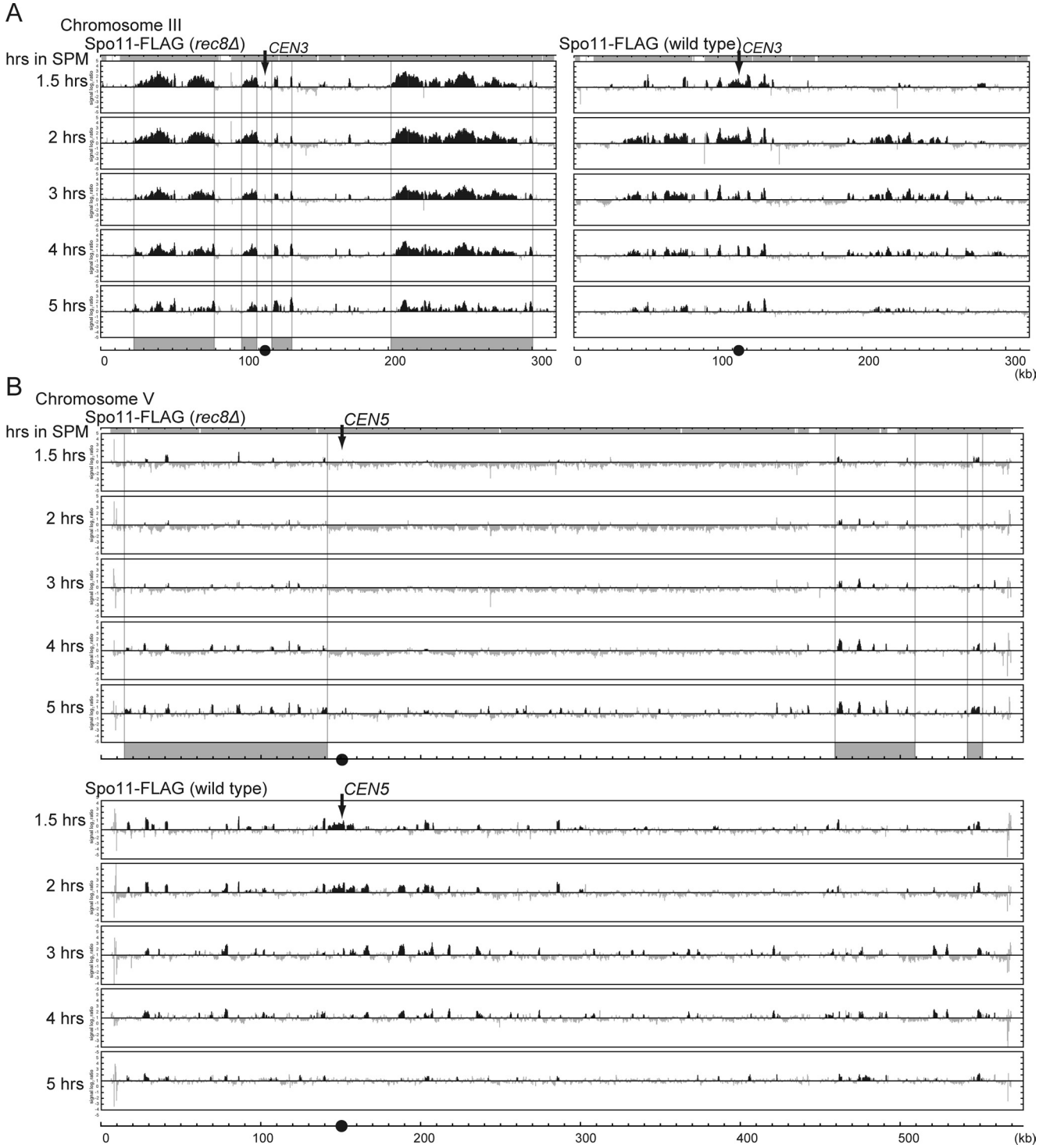

Figure 6. Effect of *REC8* deletion on the distribution of Spo11-FLAG. (A) Distribution of Spo11-FLAG on chromosome III in *rec8*Δ cells (RKD1319, left) and *REC8*$^+$ cells (RKD1311, right). Chromosome domains harboring the Spo11-FLAG binding sites in *rec8*Δ are indicated with light gray boxes on the horizontal axis. All other indications are as described in Figure 1. (B) Distribution of Spo11-FLAG on chromosome V in *rec8*Δ cells (top) and *REC8*$^+$ cells (bottom). The position of *CEN5* is indicated with arrows and closed circles.

ference in the initial localization of Spo11 and Mre11 may reflect that the chromosomal binding of Spo11 would precede that of Mre11 at distinct sites on chromosomal arms (Borde *et al*., 2004).

The HU treatment inhibited the distribution of Spo11 to later replicating regions. These results indicate that the distribution of Spo11 to later replicating regions would be coupled to DNA replication-related processes. This is consistent with previous observations that the Spo11-dependent chromatin alterations, DSB formation and DNA replication-related processes are tightly coupled (Borde *et al*., 2000; Murakami *et al*., 2003; Sasanuma *et al*., 2008; Wan *et al*., 2008).

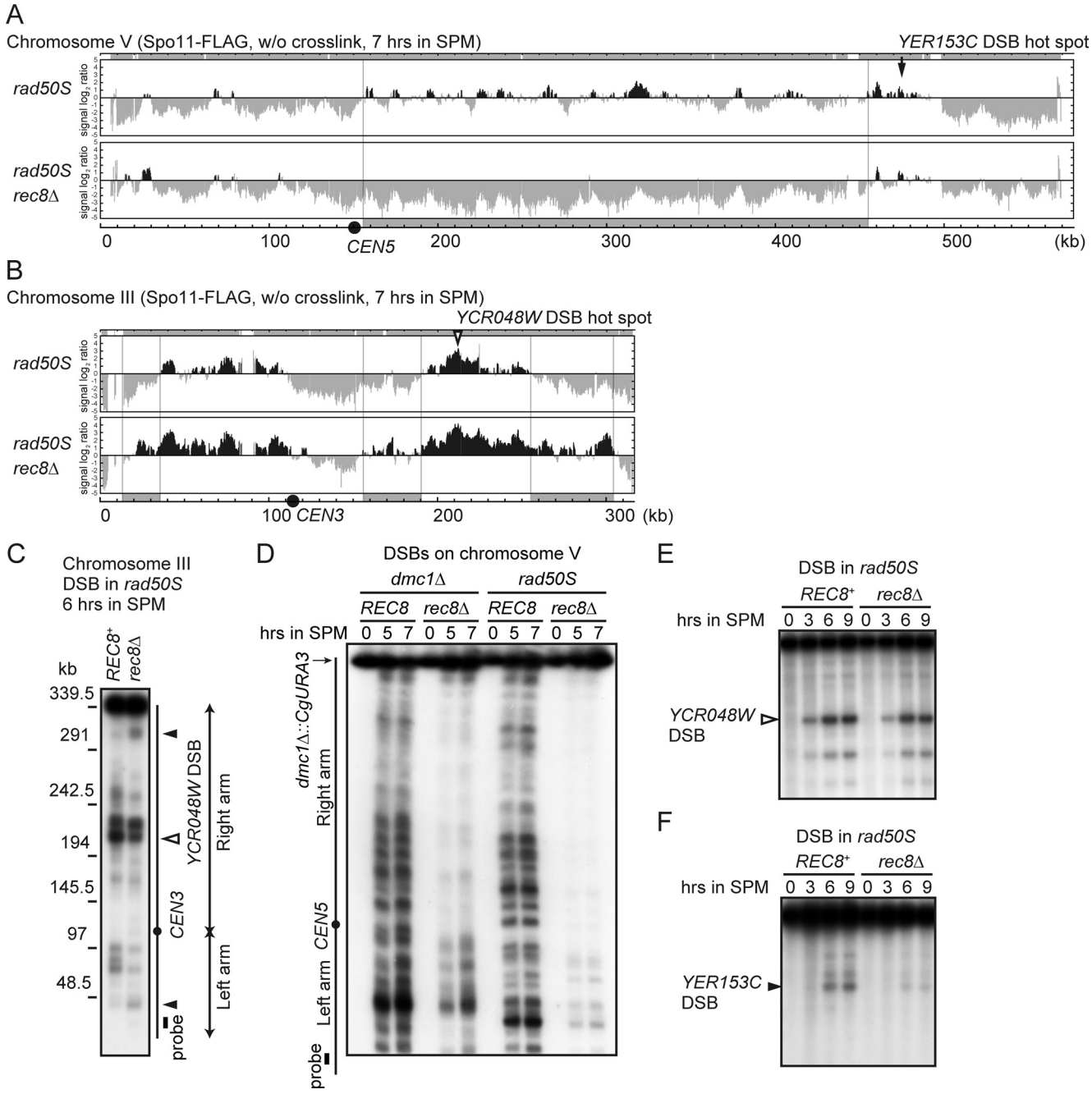

Figure 7. Effect of *REC8* deletion on the distribution of DSB sites. (A and B) DSB sites on chromosome V (A) and chromosome III (B) determined by ChIP-chip analyses using RKD1327 (*rec8*Δ *rad50S SPO11-FLAG*) and RKD1325 (*rad50S SPO11-FLAG*). (C) DSB analysis using pulsed-field gel electrophoresis followed by Southern blotting with a chromosome III left-end probe (shown in closed box). RKD1321 (*rad50S*) and RKD1323 (*rad50S rec8*Δ) were used. Closed circle indicates the centromere. (D) DSB analysis using pulsed-field gel electrophoresis followed by Southern blotting using a probe for a sequence of the chromosome V left-arm (shown in closed box). YKT23 (*dmc1*Δ), YKT28 (*dmc1*Δ *rec8*Δ), RKD1321 (*rad50S*), and RKD1323 (*rad50S rec8*Δ) were used. Closed circle indicates the centromere. (E and F) DSBs around the *YCR048W* (E) and *YER153C* (F) DSB hot spots. The DSBs were detected by Southern blotting of genomic DNA digested with BglII (*YCR048W*) or HindIII (*YER153C*). Note that the *YCR048W* DSB sites (open arrowhead) are active also *rec8*Δ, but the *YER153C* DSB site (closed arrowhead) is not.

One possible mechanism for such coupling is that the Spo11-containing DSB machineries may migrate with the active DNA replication forks. Alternatively, the dynamics of Spo11 binding to later replicating regions could be regulated by S phase checkpoints; however, this hypothesis does not fully explain the reason for the change in Spo11 distribution from early to late-replicating regions. In contrast, HU treatment of *rme1* haploid mutant is shown to cause effects on transcriptional program in some meiotic genes (Lamb and Mitchell, 2001). Thus, although Spo11 expression in normal diploids was seemingly less affected by the HU treatment in this study, we cannot exclude a possibility that altered tran-

scriptional profile in the HU-treated cells may have indirectly influenced distribution of Spo11 along meiotic chromosomes. Further investigation on the direct molecular interaction between the Spo11-containing complex and the DNA replication machineries should provide additional insight.

Dynamic distribution of Spo11 during early meiosis may be involved in meiotic chromosomal events in *S. cerevisiae*, such as premeiotic S phase progression (Cha *et al*., 2000) and centromere coupling between homologues before full level of paring (Tsubouchi and Roeder, 2005). Interestingly, bindings of Rec12 to centromeres also were observed in *S. pombe* (Ludin *et al*., 2008). These observations might imply conserved function of Spo11 during early meiosis, in addition to DSBs formation.

Role of Rec8 in the Dynamic Distribution of Spo11

The ChIP-chip data revealed that the initial binding of Spo11 to pericentromeric regions absolutely requires Rec8. This initial binding of Spo11 is likely crucial for the later distribution of Spo11 to canonical DSB sites, because the binding of Spo11 to DSB sites and the formation of meiotic DSB in the *rec8*Δ mutant were severely affected in substantial parts of most chromosomes apart from chromosome III. Interestingly, the regional *rec8*Δ effects are consistent with the observation that the deletion of $rec8^+$ in fission yeast exhibits severe defects in meiotic recombination and DSB formation in some chromosomal intervals but not in others (Ellermeier and Smith, 2005). Thus, we propose that the function of Rec8 to regulate DSB formation in a region-dependent manner would be conserved.

Then, what is the role of Rec8 in the spatial regulation of Spo11? We speculate that Rec8 may provide some molecular landmarks along the meiotic chromosome to ensure the proper distribution of Spo11/DSB sites. For example, Rec8 at centromeres may provide origins for loading of Spo11 onto the chromosome arms, whereas Rec8 at cohesion sites may provide positional and geographical information (e.g., positions of loops and axes; Blat *et al*., 2002), to coordinate loading of Spo11 onto canonical DSB sites. Rec8 at both sites may be crucial for the proper targeting of Spo11 to canonical DSB sites. Indeed, Spo11 might eventually shift from the Rec8 binding sites to chromosome loop regions including DSB sites. Such spatial transition of Spo11 may prevent recombination between sister chromatids to facilitate interhomologue recombination.

Regional Control of Spo11 Activity on Meiotic Chromosomes

Several observations, including our results, suggest that the cleavage activity of Spo11 may be differently regulated, depending upon chromosomal positions. Our ChIP-chip results revealed some regions where Spo11 bound without forming DSBs. In addition to that, DSBs were formed in cold domains (in *rad50S*) located near pericentromeric regions (Blitzblau *et al*., 2007; Buhler *et al*., 2007). Moreover, the formation of the meiosis-specific Spo11 multimer, which is essential to DSBs and requires Rec102 and Rec104 functions, has not been detected in the domains (Sasanuma *et al*., 2007; Fukuda *et al*., 2008).

One possible explanation may be that activities of meiotic DSB proteins such as Rec102, Ski8/Rec103, Rec104, Mer2/Rec107, Rec114, and Mei4 (Keeney, 2001) could be different from a region to a region. Future ChIP-chip analyses on these proteins will provide important clues to understand mechanisms pertaining to the regional regulation of meiotic DSB formation. In this regard, genome-wide analyses in our study could successfully identify most of the DSB sites observed in *dmc1*Δ mutants as well as *rad50S* mutants.

In this study, we have demonstrated that essential components of the meiotic DSB machinery are dynamically localized and that their loading on the chromosomes is controlled by mechanisms that may involve DNA replication and sister chromatid cohesion. Elucidation of such molecular mechanisms will uncover the highly integrated features of meiotic recombination initiation.

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