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# Mek1 Suppression of Meiotic Double-Strand Break Repair Is Specific to Sister Chromatids, Chromosome Autonomous and Independent of Rec8 Cohesin Complexes

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ABSTRACT

During meiosis, recombination is directed to occur between homologous chromosomes to create connections necessary for proper segregation at meiosis I. Partner choice is determined at the time of strand invasion and is mediated by two recombinases: Rad51 and the meiosis-specific Dmc1. In budding yeast, interhomolog bias is created in part by the activity of a meiosis-specific kinase, Mek1, which is localized to the protein cores of condensed sister chromatids. Analysis of meiotic double-strand break (DSB) repair in haploid and disomic haploid strains reveals that Mek1 suppresses meiotic intersister DSB repair by working directly on sister chromatids. Rec8 cohesin complexes are not required, however, either for suppression of intersister DSB repair or for the repair itself. Regulation of DSB repair in meiosis is chromosome autonomous such that unrepaired breaks on haploid chromosomes do not prevent interhomolog repair between disomic homologs. The pattern of DSB repair in haploids containing Dmc1 and/or Rad51 indicates that Mek1 acts on Rad51-specific recombination processes.

In eukaryotes, meiosis is a specialized type of cell division that produces the gametes required for sexual reproduction. In meiosis, one round of DNA replication is followed by two rounds of chromosome segregation, termed meiosis I and II. As a result of the two divisions, four haploid cells are produced, each containing half the number of chromosomes as the diploid parent. Proper segregation at meiosis I requires connections between homologous chromosomes that are created by a combination of sister chromatid cohesion and recombination (Petronczki *et al.* 2003). In vegetative cells, cohesion is mediated by multisubunit ring-shaped complexes that are removed by proteolysis of the kleisin subunit, Mcd1/Scc1 (Onn *et al.* 2008). In meiotic cells, introduction of a meiosis-specific kleisin subunit, Rec8, allows for a two-step removal of cohesion with loss of arm cohesion at anaphase I and centromere cohesion at anaphase II (Klein *et al.* 1999). Missegregation of chromosomes during meiosis causes abnormal chromosome numbers in gametes that may lead to infertility and genetic disorders such as trisomy 21 or Down's syndrome.

In mitotically dividing budding yeast cells, recombination is mediated by an evolutionarily conserved RecA-like recombinase, Rad51, and occurs preferentially between sister chromatids (Kadyk and Hartwell 1992). In contrast, recombination during meiosis is initiated by the deliberate formation of double-strand breaks (DSBs) by an evolutionarily conserved, topoisomerase-like protein, Spo11, and occurs preferentially between homologous chromosomes (Jackson and Fink 1985; Schwacha and Kleckner 1997; Keeney 2001). After DSB formation, the 5′ ends on either side of the breaks are resected, resulting in 3′ single stranded (ss) tails. Rad51, and the meiosis-specific recombinase Dmc1, bind to the 3′ ssDNA tails to form protein/DNA filaments that promote strand invasion of homologous chromosomes. DNA synthesis and ligation result in the formation of double Holliday junctions, which are then preferentially resolved into crossovers (Allers and Lichten 2001; Hunter 2007).

The precise roles that the Rad51 and Dmc1 recombinase activities play in meiotic recombination have been unclear because experiments have indicated both overlapping and distinct functions for the two proteins (Sheridan and Bishop 2006; Hunter 2007). While both *rad51*Δ and *dmc1*Δ mutants reduce interhomolog recombination, other studies suggest that Rad51, in complex with the accessory protein Rad54, is involved primarily in intersister DSB repair. In contrast, Dmc1, in conjunction with the accessory protein Rdh54/Tid1 (a paralog of Rad54), effects DSB repair in meiotic cells by invasion of nonsister chromatids (Dresser *et al.* 1997; Schwacha and Kleckner 1997; Shinohara *et al.* 1997a,b; Arbel *et al.* 1999; Bishop *et al.* 1999; Hayase *et al.* 2004; Sheridan and Bishop 2006).

The preference for recombination to occur between homologous chromosomes during meiosis is created in

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part by Dmc1. DSBs accumulate in *dmc1*Δ diploids due to a failure in strand invasion (Bishop *et al.* 1992; Hunter and Kleckner 2001). In the efficiently sporulating SK1 strain background, these unrepaired breaks trigger the meiotic recombination checkpoint, resulting in prophase arrest (Lydall *et al.* 1996; Roeder and Bailis 2000). In *dmc1*Δ mutants, Rad51 is present at DSBs, yet there is no strand invasion of sister chromatids (Bishop 1994; Shinohara *et al.* 1997a). These results suggest that in addition to Dmc1 promoting interhomolog strand invasion, Rad51 activity must also be suppressed.

Recent studies have shown that during meiosis Rad51 recombinase activity is inhibited by two different mechanisms that decrease the formation of Rad51/Rad54 complexes: (1) binding of the meiosis-specific Hed1 protein to Rad51, thereby excluding interaction with Rad54, and (2) reduction in the affinity of Rad54 for Rad51 due to phosphorylation of Rad54 by Mek1 (Tsubouchi and Roeder 2006; Busygina *et al.* 2008; Niu *et al.* 2009). Mek1 is a meiosis-specific kinase that is activated in response to DSBs (Niu *et al.* 2005, 2007; Carballo *et al.* 2008). In addition to phosphorylating Rad54, Mek1 phosphorylation of an as yet undetermined substrate is required to suppress Rad51/Rad54-mediated strand invasion of sister chromatids (Niu *et al.* 2009).

To dissect the mechanism by which Mek1 suppresses meiotic intersister DSB repair, we took advantage of the ability of yeast cells to undergo haploid meiosis. The lack of homologous chromosomes in haploid cells makes it possible to examine sister-chromatid-specific events in the absence of interhomolog recombination. De Massy *et al.* (1994) previously observed a delay in DSB repair in haploid cells and proposed that this delay was due to a constraint in using sister chromatids. We have shown that this delay is dependent on *MEK1* and utilized the haploid system to determine various biological parameters required to suppress meiotic intersister DSB repair. Our results indicate that Rad51 and Dmc1 recombinase activities have distinct roles during meiosis and that interhomolog bias is established specifically on sister chromatids through regulation of Rad51, not Dmc1. *rec8*Δ diploids exhibit defects in meiotic DSB repair (Klein *et al.* 1999; Brar *et al.* 2009). Given that cohesin complexes are specific for sister chromatids, we investigated the role of *REC8* in intersister DSB repair and found it is required neither for suppressing intersister DSB repair during meiosis nor for the repair itself.

## MATERIALS AND METHODS

**Plasmids:** The plasmid, pDT20, contains a 0.6-kb sequence of chromosome VII (coordinates 497,700–497,759) and was created by amplifying a fragment using genomic DNA and primers that engineered *Sac*I and *Sph*I sites onto the ends. After digestion, the fragment was subcloned into *Sac*I/*Sph*I-digested pVZ1 (Hollingsworth and Johnson 1993). Chromosome III hotspot probes were derived from pME1210 (*YCR048w*) (Woltering *et al.* 2000) and pNH90 (*HIS4/LEU2*) (Hunter and Kleckner 2001). The chromosome VI hotspot (*HIS2*) was detected using pH 21 (Bullard *et al.* 1996) (provided by Bob Malone) and the chromosome VIII hotspot (*ARG4*) used pMJ77 (provided by Michael Lichten). The *mek1-as* allele in pJR2 was constructed by subcloning a 3.2-kb *Eco*RI/*Sal*I fragment from pB131-Q241G (Niu *et al.* 2009) into *Eco*RI/*Sal*I-digested Ylp5 (Parent *et al.* 1985). pRS306 is a *URA3* integrating plasmid (Sikorski and Hieter 1989).

**Yeast strains and media:** All strains are derived from the SK1 background, except for NH705-32-1 dmc1, which is from the A364a background. The genotypes of each strain can be found in Table 1. Liquid and solid media were as described previously (Vershon *et al.* 1992; De Los Santos and Hollingsworth 1999). *SIR2* and *RME1* were deleted with *natMX4*, using the polymerase chain reaction (PCR) method of Tong and Boone (2005). *MEK1* was mutated using pTS21 (*mek1*Δ*::URA3*), pTS1 (*mek1*Δ*::LEU2*) (De Los Santos and Hollingsworth 1999), or *natMX4*. *REC8* and the second exon of *DMC1* were deleted with *kanMX6*, using the PCR method of Longtine *et al.* (1998). All deletions were confirmed by yeast colony PCR. pRS306 was targeted to integrate at *ura3* by digestion with *Stu*I while pJR2 was integrated downstream of the *MEK1* open reading frame by digestion with *Rsr*II. NH716 is a diploid resulting from a cross between NHY1215 and NHY1210 (provided by N. Hunter).

The chromosome III disome, Kar-3-WT was constructed using a "kar cross" (Dutcher 1981). Strains carrying *kar1-1* fail to efficiently undergo karyogamy, creating cells with two nuclei. At low frequency, chromosomes in these dikaryons can be transferred from one nucleus to the other. Disomic III haploids can be obtained by selecting for recessive resistance markers carried by chromosomes in the recipient nucleus as well as for prototrophic markers carried on chromosome III from the donor cell (Figure 1). Our recipient strain, NHY1215 $Can^R Cyh^R$ was generated by the sequential selection for *can1* and *cyh2* mutants on SD −Arg + 60 μg/ml canavanine and YPDcom + 10 μg/ml cycloheximide, respectively. For the donor strain, *DMC1* was first deleted with *natMX4* to introduce a dominant drug resistance marker. Putative chromosome III disomic haploids were tested for heterozygosity at the *MAT* locus by screening for nonmaters. In *kar1-1* crosses, ~10% of the cells are diploid (Dutcher 1981). The possibility that Kar-3-WT is diploid was ruled out by the following:

1. Selecting for two recessive resistance markers: The donor strain was *CAN1 CYH2* and therefore the diploid should be sensitive to both canavanine and cycloheximide.
2. Assaying for nourseothricin (NAT) sensitivity: The donor strain was *dmc1*Δ*::natMX4*. Since $Nat^R$ is dominant, the diploid can grow on SD + NAT plates, while the disomic haploid cannot.
3. Quantitation of the number of chromosomes by Southern blot.

Plugs were made from 5-ml YEPD stationary cultures of the NHY1215 sir2 haploid, the diploid NH929, and the disomic haploid Kar-3-WT, as described by Borde *et al.* (1999). In addition, a *sir2*Δ*::natMX4* derivative of Kar-3-WT, Kar-3-sir2, was also examined. The chromosomes were fractionated using a 1.5% contoured-clamp homogeneous electric field (CHEF) gel. After transfer to a nylon membrane, the blot was probed simultaneously with radioactive probes derived from sequences on chromosome III (0.9-kb *Hin*dIII fragment from pME1210) and chromosome VII sequences (0.6-kb *Sac*I/*Sph*I fragment from pDT20). The amount of radioactive labeling of each chromosome was quantitated using the Multigauge

**TABLE 1**

***Saccharomyces cerevisiae* strains**

| Name | Genotype | Source |
|---|---|---|
| NHY1215 | *MAT*α *leu2::hisG his4-X::LEU2-(NgoMIV) ho::hisG ura3(Δpst-sma)* | N. Hunter |
| NHY1215 sir2 | NHY1215 only *sir2Δ::natMX4* | This work |
| NHY1215 sir2 mek1 | NHY1215 only *mek1Δ::URA3 sir2Δ::natMX4* | This work |
| NHY1215 sir2 dmc1 | NHY1215 only *dmc1Δ::kanMX6 sir2Δ::natMX4* | This work |
| NHY1215 sir2 dmc1 mek1 | NHY1215 only *mek1Δ::URA3 dmc1Δ::kanMX6 sir2Δ::natMX4* | This work |
| NHY1215 sir2 rec8 | NHY1215 only *sir2Δ::natMX4 rec8Δ::kanMX6* | This work |
| NHY1215 sir2 mek1 rec8 | NHY1215 only *sir2Δ::natMX4 rec8Δ::kanMX6 mek1Δ::URA3* | This work |
| NHY1215 rme1 | NHY1215 only *rme1Δ::natMX4* | This work |
| NHY1215 rme1 mek1 | NHY1215 only *mek1Δ::URA3 rme1Δ::natMX4* | This work |
| NHY1215 rme1 dmc1 | NHY1215 only *dmc1Δ::kanMX6 rme1Δ::natMX4* | This work |
| NHY1215 rme1 dmc1 mek1 | NHY1215 only *mek1Δ::URA3 dmc1Δ::kanMX6 rme1Δ::natMX4* | This work |
| NHY1215 sir2 rad52 | NHY1215 only *sir2Δ::natMX4 rad52Δ::kanMX6* | This work |
| NHY1215 can1 cyh2 | NHY1215 only can1 cyh2 | This work |
| NH716[a] | $\frac{MAT\alpha\ leu2::hisG\ his4\text{-}X::LEU2\,(NgoMIV)}{MAT\mathbf{a}\ leu2::hisG\ HIS4::LEU2}\ \frac{ho\Delta::hisG}{ho\Delta::hisG}\ \frac{ura3(\Delta pst\text{-}sma)}{ura3(\Delta pst\text{-}sma)}$ | N. Hunter |
| NH729 | NH716 only $\frac{mek1\Delta::natMX4}{mek1\Delta::natMX4}$ | This work |
| NH705-32-1 dmc1 | *MAT***a** *ura3-52 kar1-1 ade2 dmc1Δ::natMX4* | This work |
| Kar-3-WT | $\frac{MAT\alpha\ leu2::hisG\ his4\text{-}X::LEU2(NgoMIV)}{MAT\mathbf{a}\ LEU2\quad HIS4}\ \frac{ho::hisG}{}\ \frac{ura3(\Delta pst\text{-}sma)\ can1}{}\ \frac{cyh2}{}$ | This work |
| Kar-3-sir2 | Kar-3-WT only *sir2Δ::natMX4* | This work |
| Kar-3-mek1 | Kar-3-WT only *mek1Δ::URA3* | This work |
| NH929 | $\frac{MAT\alpha\ leu2::hisG\ his4\text{-}X::LEU2\,(NgoMIV)}{MAT\mathbf{a}\ LEU2\quad HIS4}\ \frac{ho::hisG}{ho::hisG}\ \frac{ura3(\Delta pst\text{-}sma)}{ura3(\Delta pst\text{-}sma)}\ \frac{can1\,cyh2}{can1\,cyh2}$ | This work |
| NH144[a] | $\frac{MAT\alpha\ leu2\text{-}k\quad HIS4}{MAT\mathbf{a}\ leu2::hisG\ his4\text{-}X}\ \frac{arg4\text{-}Nsp}{ARG4}\ \frac{ura3}{ura3}\ \frac{lys2}{lys2}\ \frac{ho\Delta::LYS2}{ho\Delta::LYS2}$ | Hollingsworth *et al.* (1995) |
| NH746 | NH144 only $\frac{rec8\Delta::kanMX6}{rec8\Delta::kanMX6}$ | This work |
| NH748 | NH144 only $\frac{dmc1\Delta::natMX4}{dmc1\Delta::natMX4}$ | This work |
| NH748::pRS306 | NH144 only $\frac{ura3::URA3}{ura3}\ \frac{dmc1\Delta::natMX4}{dmc1\Delta::natMX4}$ | This work |
| NH749 | NH144 only $\frac{dmc1\Delta::natMX4}{dmc1\Delta::natMX4}\ \frac{mek1\Delta::LEU2}{mek1\Delta::LEU2}$ | This work |
| NH749::pJR2 | NH144 only $\frac{dmc1\Delta::natMX4}{dmc1\Delta::natMX4}\ \frac{mek1\Delta::LEU2::URA3::mek1\text{-}as}{mek1\Delta::LEU2}$ | This work |
| NH751 | NH144 only $\frac{rec8\Delta::kanMX6}{rec8\Delta::kanMX6}\ \frac{mek1\Delta::LEU2}{mek1\Delta::LEU2}$ | This work |
| NH752::pRS306 | NH144 only $\frac{ura3::URA3}{ura3}\ \frac{dmc1\Delta::natMX4}{dmc1\Delta::natMX4}\ \frac{rec8\Delta::kanMX6}{rec8\Delta::kanMX6}$ | This work |
| NH753 | NH144 only $\frac{dmc1\Delta::natMX4}{dmc1\Delta::natMX4}\ \frac{rec8\Delta::kanMX6}{rec8\Delta::kanMX6}\ \frac{mek1\Delta::LEU2}{mek1\Delta::LEU2}$ | This work |
| NH753::pJR2 | NH144 only $\frac{dmc1\Delta::natMX4}{dmc1\Delta::natMX4}\ \frac{rec8\Delta::kanMX6}{rec8\Delta::kanMX6}\ \frac{mek1\Delta::LEU2::URA3::mek1\text{-}as}{mek1\Delta::LEU2}$ | This work |

[a] Although the haploid parents of NH716 and NH144 are derived from the SK1 background, they were obtained from different sources and are not necessarily isogenic with each other.

Software and a Fujifilm FLA 7000 phosphoimager and the ratio of chromosome III/chromosome VII hybridization was calculated. This ratio was the same in the diploid and haploid strains, 0.7 and 0.8, respectively. In contrast, the chromosome III disomic haploids Kar-3-WT and Kar-3-sir2 exhibited ratios that were approximately twofold higher (1.4 and 1.5, respectively), as expected if there are two copies of chromosome III to a single copy of chromosome VII.

To construct a diploid that is isogenic with Kar-3-WT, a haploid derivative that had lost the *MAT*α chromosome was isolated by screening for colonies that mated as "**a**" cells. This *MAT***a** Kar-3-WT derivative was then crossed with NHY1215 $Can^R Cyh^R$ to generate NH929 (Figure 1).

**DSB analysis:** For each time point plugs were prepared and the DNA was digested *in situ* as described in Borde *et al.* (1999). The exception was the experiment shown in Figure 4,

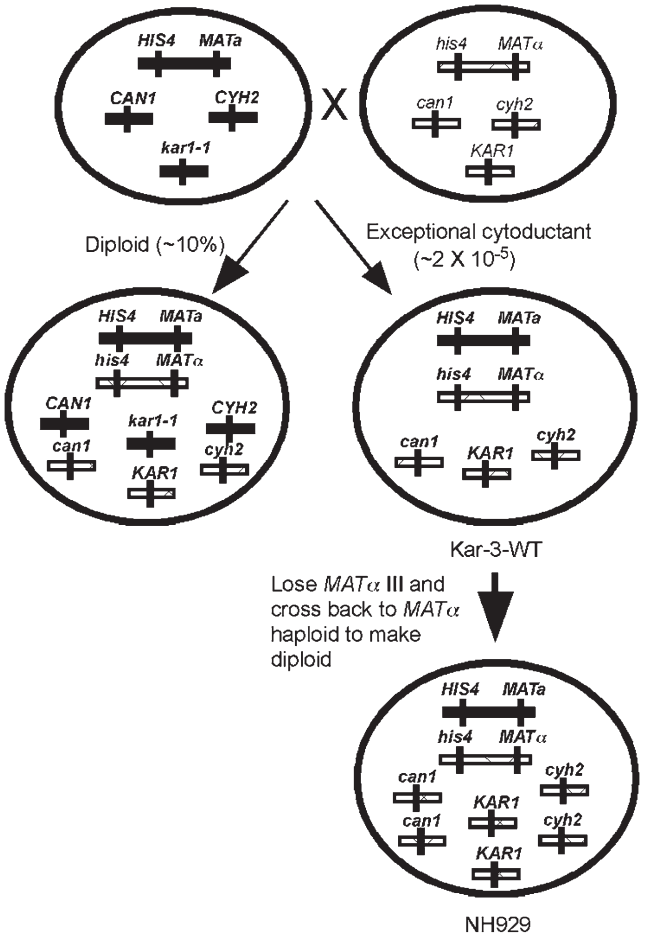


Figure 1.—Schematic of the construction of the disomic haploid, Kar-3-WT and its isogenic diploid. A *MAT***a** *kar1-1* donor strain was crossed to a *MAT*α *his4* recipient strain and exceptional cytoductants containing the haploid genome of the recipient strain and chromosome III from the donor strain were selected to generate the disomic haploid, Kar-3-WT. A diploid isogenic with Kar-3-WT was created by losing the chromosome carrying *MAT*α *his4* and backcrossing the resulting *MAT***a** *HIS4* haploid to the *MAT*α *his4* parent to make NH929.

in which DNA was crosslinked with psoralen and then isolated from cells prior to restriction enzyme digestion as described in Oh *et al.* (2009). The chromosome III hotspot, *YCR048w*, was monitored using a *Bgl*II genomic digest and a 0.9-kb *Hin*dIII fragment from pME1210 (Wu and Lichten 1994); for *HIS4/LEU2*, a *Xho*I digest and a 0.6-kb *Age*I/*Bgl*II fragment from pNH90 were used (Hunter and Kleckner 2001). The chromosome VI *HIS2* hotspot was detected with a *Bgl*II digest and a 1-kb *Bgl*II/*Eco*RI fragment from pH21 (Bullard *et al.* 1996). The chromosome VIII *ARG4* hotspot also used a *Bgl*II genomic digest and a 0.6-kb *Hpa*I/*Eco*RV fragment from pMJ77. The plugs were loaded onto 0.8% agarose gels that were run for 24 hr in 1× TBE buffer at 4° at 90 V for *YCR048w* and *HIS2* and 70 V for *ARG4*. To detect the *HIS4/LEU2* DSBs, 0.6% agarose gels were run at 70 V at room temperature. DSBs were quantified using the Image Quant 1.1 software and a Molecular Dynamics Phosphoimager or the Multi-Gauge Software with a FujiFilm FLA 7000 Phosphoimager.

**Time courses:** Liquid sporulation was performed at 30° in 2% potassium acetate at a density of $3 \times 10^7$ cells/ml. Ten-milliliter samples were taken at the indicated times, mixed with 50 mm EDTA and 10 ml 95% ethanol, and stored at −20°. Meiotic progression was monitored by staining nuclei with 4′,6-diamidino-2-phenylindole (DAPI) and using fluorescence microscopy to score binucleate cells (meiosis I) and tetranucleate cells (meiosis II). For each strain at each time point, 200 cells were counted. Every time course was performed at least twice.

## RESULTS

***MEK1*-dependent suppression of intersister DSB repair does not require the presence of homologous chromosomes:** To test whether Mek1 suppression of meiotic intersister DSB repair is specific to sister chromatids, DSBs were examined in haploid cells where no homologs are available. If suppression of intersister repair requires homologous chromosomes, then DSBs should be repaired in *dmc1*Δ haploid strains, even though Mek1 is active. Alternatively, if the suppression mechanism is confined to sister chromatids, haploid *dmc1*Δ strains should exhibit unrepaired DSBs.

These two possibilities were distinguished by analyzing meiotic DSB repair in *dmc1*Δ haploids at the *YCR048w*, *HIS2*, and *ARG4* hotspots, located on chromosomes III, VI, and VIII, respectively. To enable haploid cells to enter meiosis, *SIR2* was deleted, thereby allowing *MAT***a** and *MAT*α information to be expressed from the normally silent mating-type loci (Rine and Herskowitz 1987). In the *sir2*Δ *dmc1*Δ haploid, DSBs appeared by 4 hr at all three hotspots and persisted up to 12 hr (Figure 2A). The DSBs in the *dmc1*Δ haploid resemble those in *dmc1*Δ diploids in that they accumulate and become hyperresected (Bishop *et al.* 1992). Deletion of *MEK1* results in efficient repair of DSBs at all three locations (Figure 2A). The reduced number of DSBs observed in the *mek1*Δ and *mek1*Δ *dmc1*Δ haploids is likely due to rapid repair using sister chromatids, as opposed to a decrease in DSB formation, because *mek1*Δ diploids have previously been shown to exhibit wild-type DSB levels when processing of the breaks is prevented (Pecina *et al.* 2002). These data indicate that the inhibition of DSB repair observed in *dmc1*Δ haploids requires *MEK1*, similar to what is observed in diploid cells (Xu *et al.* 1997; Wan *et al.* 2004).

*RME1* is a haploid-specific gene that encodes a protein that negatively regulates entry into meiosis by repressing *IME1*, a transcription factor required for the onset of meiosis (Mitchell and Herskowitz 1986; Kassir *et al.* 1988). *RME1* is repressed by the a1/α2 transcription factor and this repression is the reason that cells must normally be heterozygous for mating type to sporulate (Covitz *et al.* 1991). *rme1*Δ mutants bypass the requirement for a1/α2 and therefore this mechanism for inducing haploid meiosis is completely independent of the *sir2*Δ mechanism. Similar to *sir2*Δ *dmc1*Δ, DSBs accumulated in the *rme1*Δ *dmc1*Δ haploid and were repaired in the *rme1*Δ *dmc1*Δ *mek1*Δ strain, indicating that the *MEK1*-dependent suppression of in-

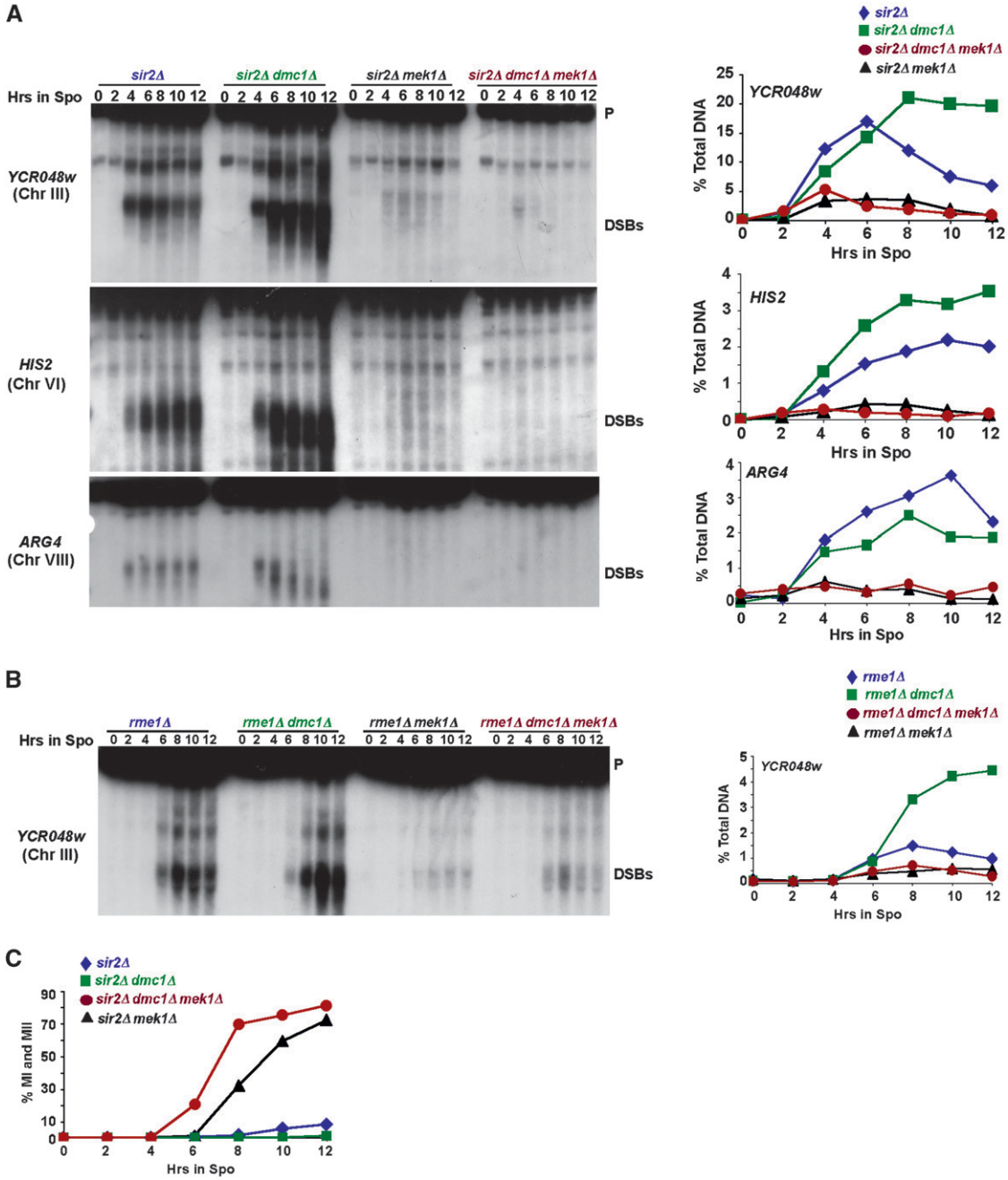


FIGURE 2.—Suppression of meiotic intersister DSB repair in various haploids. (A) Isogenic derivatives of NHY1215 containing *sir*2Δ, *sir*2Δ *dmc1*Δ, *sir2*Δ *mek1*Δ, or *sir*2Δ *dmc1*Δ *mek1*Δ were sporulated at 30°. DSBs at three different hotspots were analyzed at various times after transfer to Spo medium. (B) Similar experiment to those in A, only the NHY1215 derivatives contain *rme1*Δ instead of *sir2*Δ. Graphs indicate the percentage of total DNA constituted by the DSB fragments. (C) Meiotic progression of the time courses shown in A. "% MI and MII" refers to the numbers of bi- and tetranucleate cells, respectively.

tersister repair is a general property of meiotic haploid cells, and not a function of *sir2*Δ mutants (Figure 2B). Therefore the mechanism by which *MEK1* suppresses intersister repair is specific to sister chromatids.

**Meiotic DSB repair in *DMC1* haploid cells is also dependent on *MEK1*:** DSB repair is delayed or absent in the *sir2*Δ and *rme1*Δ haploids (Figure 2, A and B; DE MASSY *et al.* 1994). Elimination of *MEK1* from these strains results in efficient repair of these breaks, similar to the *dmc1*Δ *mek1*Δ haploids (Figure 2, A and B). Therefore Mek1 is able to suppress intersister DSB repair in haploid cells even when Dmc1 is present.

In diploid cells, a failure to repair DSBs triggers the meiotic recombination checkpoint and results in prophase arrest (LYDALL *et al.* 1996; ROEDER and BAILIS 2000). Meiotic progression is delayed or absent in *sir2*Δ *DMC1* and *sir2*Δ *dmc1*Δ haploids, respectively, but not in *sir2*Δ *mek1*Δ or *sir2*Δ *dmc1*Δ *mek1*Δ, indicating

unrepaired breaks are effective in activating the recombination checkpoint even in the absence of homologous chromosomes (Figure 2C).

**The regulation of meiotic DSB repair is chromosome autonomous:** There are a number of possible explanations for the inefficient DSB repair observed in the *sir2*Δ and *rme1*Δ haploids. One possibility is that DSB repair is normally coordinated between chromosomes. For example, there could be a checkpoint that delays repair until all chromosomes are homologously paired or have initiated strand invasion between homologs. This idea was tested by examining meiotic DSB repair in haploid strains containing two copies of chromosome III. If DSB repair between different chromosomes is coordinated, then the broken haploid chromosomes should inhibit DSB repair between the disomic chromosome III homologs. If, however, meiotic DSB repair is chromosome autonomous, then DSBs on the disomic chromosome should be fixed by interhomolog recombination, while the breaks on the haploid chromosomes remain unrepaired.

A chromosome III disomic haploid and isogenic diploid were created as described in *Materials and Methods* (Figure 1). The *YCR048w* and *HIS4/LEU2* hotspots on chromosome III were used to look at DSB repair on the disomic chromosome. The *HIS4/LEU2* hotspot has the advantage that interhomolog recombination can be directly monitored by physical assays (Hunter and Kleckner 2001). After 12 hr in sporulation medium, DSBs disappeared at both hotspots in the diploid and disomic haploid strains, but not in the haploid (Figure 3, A and B). Restriction fragments indicative of crossovers were seen in both the wild-type and the *sir2*Δ disomic haploids, confirming that interhomolog recombination occurred (Figure 3A). The number of crossovers in the disome is delayed and reduced relative to that in the diploid, however. This delay is not due to *sir2*Δ, since the wild-type disome behaved similarly. The *HIS2* hotspot on chromosome VI is present in only one copy in the disomic haploid. DSBs at this hotspot failed to get efficiently repaired in both the disomic haploid and the haploid strains, and both strains were delayed/arrested in meiotic prophase (Figure 3, C and D). Deletion of *MEK1* relieved the progression defect of these strains and allowed repair of the *HIS2* breaks (data not shown). Therefore repair of DSBs on different chromosomes occurs independently of each other.

**Dmc1 is capable of intersister DSB repair in haploid cells:** Another explanation for the delay/absence of DSB repair in wild-type haploids is that Mek1 acts directly on Dmc1 to suppress strand invasion of sister chromatids. To remove any regulation that might be provided by Rad51, filaments containing only Dmc1 were created by deletion of *RAD52*, a mediator protein that is required for loading Rad51 onto the breaks (Lao *et al.* 2008). [This indirect method of preventing Rad51 from assembling onto breaks is necessary because *rad51*Δ mutants prevent efficient loading of Dmc1 (Bishop 1994; Shinohara *et al.* 1997a)]. In contrast to the DSBs in the wild-type and *dmc1*Δ haploids that persisted up to 10 hr, some of the DSBs in the *rad52*Δ haploid disappeared, indicating that Dmc1 can mediate strand invasion of sister chromatids even when Mek1 is active (Figure 4A). The *rad52*Δ cells failed to enter meiosis I, however, suggesting that a fraction of the DSBs were not repaired (Figure 4B). These results demonstrate that Mek1 does not suppress Dmc1 directly, but rather that it is the presence of Rad51 that constrains Dmc1 from interacting with sister chromatids. Furthermore, they rule out the idea that Dmc1 is activated by the presence of homologous chromosomes.

**Meiotic intersister DSB repair occurs independently of *REC8*:** To determine whether meiotic cohesin complexes containing Rec8 are necessary for intersister DSB repair, DSBs were compared in *rec8*Δ and *mek1*Δ *rec8*Δ haploids. *rec8*Δ differentially affects the recruitment of Spo11 to chromosomes such that few to no breaks are observed on chromosomes such as VI and VIII (ruling out examination of the *HIS2* and *ARG4* hotspots), while chromosome III is less affected (Kugou *et al.* 2009). To see whether *REC8* is required for meiotic intersister recombination, DSB repair was therefore monitored at *YCR048w* and *HIS4/LEU2* in *sir2*Δ *rec8*Δ and *sir2*Δ *mek1*Δ *rec8*Δ haploids. DSBs accumulated and became hyperresected in the *sir2*Δ *rec8*Δ haploid at both hotspots, similar to the *sir2*Δ haploid (Figure 5A). No meiotic progression was observed in the *sir2*Δ *rec8*Δ strain, indicating that *rec8*Δ is not directly required for the meiotic recombination checkpoint. Deletion of *MEK1* in the *rec8*Δ mutant resulted in repair of the DSBs and the progression of the cells through the meiotic divisions (Figure 5, A and C). Rec8 cohesin complexes therefore are not required for sister-based repair.

*rec8*Δ exhibits a significant fraction of unrepaired breaks at the *YCR048w* hotspot in diploid cells (Klein *et al.* 1999; Brar *et al.* 2009) (Figure 5B). The accumulation of DSBs is not as high as in *dmc1*Δ diploids, perhaps because of less efficient recruitment of Spo11. Consistent with the haploid experiment, DSB repair and meiotic progression were observed in *mek1*Δ *rec8*Δ and *dmc1*Δ *mek1*Δ *rec8*Δ dipioids (Figure 5, B and D). Interestingly, DSB repair is less efficient and meiotic progression delayed in *dmc1*Δ *mek1*Δ *rec8*Δ strains relative to *dmc1*Δ *mek1*Δ. Therefore although *REC8* is not required for repair using sister chromatids, it does promote such repair. The *MEK1*-dependent accumulation of DSBs in *rec8*Δ and *dmc1*Δ *rec8*Δ strains rules out Rec8 as the target of Mek1 responsible for suppressing intersister DSB repair.

***REC8* functions with *MEK1* to activate the meiotic recombination checkpoint:** Mek1-as is an analog-sensitive version of Mek1 that can be inhibited by addition of purine analogs to the sporulation medium (Wan *et al.* 2004; Niu *et al.* 2005). Genetic experiments monitoring spore

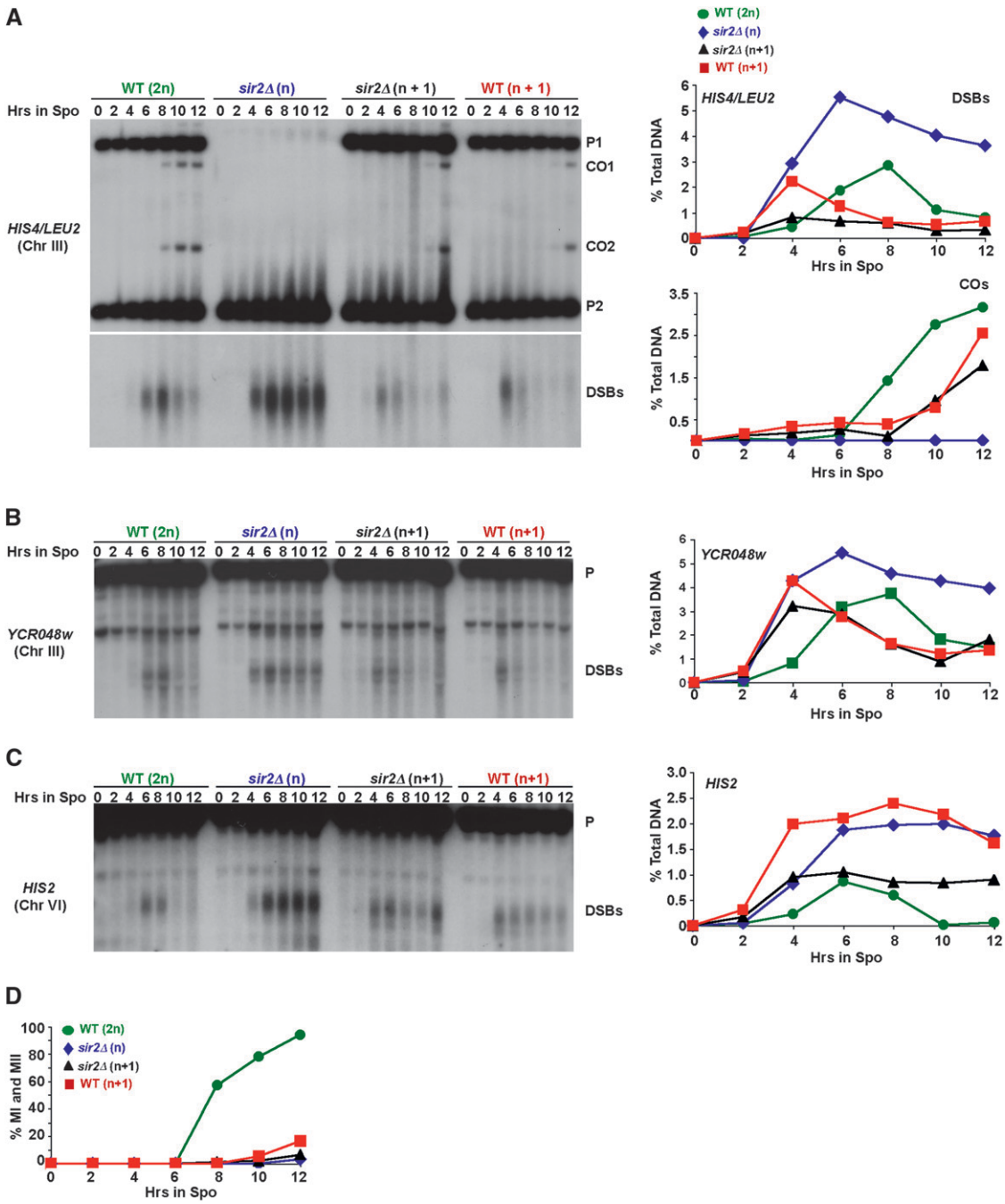


FIGURE 3.—Meiotic DSB repair in diploid, haploid, and disomic haploid strains. Meiotic time courses of isogenic diploid (2*n*, NH929), haploid (*n*, NHY1215 sir2), and chromosome III disomic haploid (*n* + 1, Kar-3-sir2 and Kar-3-WT) strains were performed. (A) DSBs and crossovers at the *HIS4/LEU2* hotspot on chromosome III. Parental (P) bands are indicated as P1 and P2 and crossover (CO) bands are indicated as CO1 and CO2. COs and DSBs were detected on the same blot but for clarity, a longer exposure of the DSB portion of the blot is shown. (B) DSBs at the *YCR048w* hotspot on chromosome III. (C) DSBs at the *HIS2* hotspot on chromosome VI. Graphs indicate quantitation of the DSBs and COs. (D) Meiotic progression of NH929, NHY1215 sir2, Kar-3-sir2, and Kar-3-WT measured by counting DAPI-stained nuclei.

viability and meiotic arrest in *dmc1*Δ diploids indicated that *mek1-as* is as functional as wild-type *MEK1 in vivo*, although kinase assays revealed that Mek1-as has a reduced affinity for ATP *in vitro* (WAN *et al.* 2004; NIU *et al.* 2009). A single copy of *mek1-as* was integrated into a *mek1*Δ *dmc1*Δ diploid isogenic to the *MEK1 dmc1*Δ strain shown in Figure 6. In contrast to other *mek1-as* diploids we have constructed, this diploid exhibited ~40% meiotic progression and a reduction in the number of DSBs at 10 hr at the *YCR048w* hotspot (Figure 6). (Note that these experiments were carried out in the absence of inhibitor and Mek1-as should therefore be active). This result suggests that in this particular derivative of SK1, a single copy of *mek1-as* provides less kinase activity *in vivo*

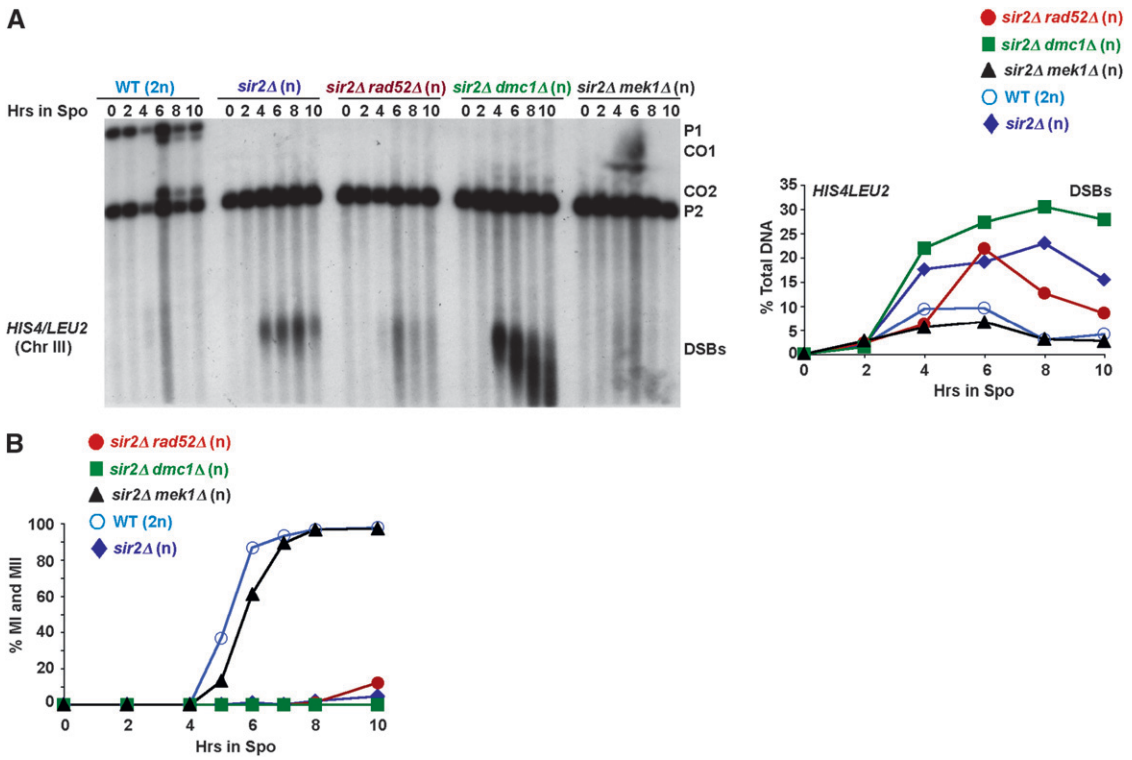


FIGURE 4.—Differential patterns of DSB repair in wild-type haploids compared to *dmc1*Δ or *rad52*Δ haploids. (A) Diploid wild-type strain NH716 and haploid strains *sir2*Δ (NHY1215 sir2), *sir2*Δ *rad52*Δ (NHY1215 sir2 rad52), *sir2*Δ *dmc1*Δ (NHY1215 sir2 dmc1), and *sir2*Δ *mek1*Δ (NHY1215 sir2 mek1) were sporulated at 30° and analyzed at the indicated time points for DSBs at the *HIS4/LEU2* hotspot. Graph indicates quantitation of DSBs. (B) Meiotic progression of the time courses shown in A.

than wild type. DSBs accumulate in the *dmc1*Δ rec8Δ diploid, confirming that *REC8* is not required for suppressing intersister DSB repair (Figure 6A). When *mek1-as* was combined with *rec8*Δ *dmc1*Δ, meiotic progression occurred with wild-type kinetics and efficiency, compared to *dmc1*Δ and *rec8*Δ *dmc1*Δ, even though substantial numbers of DSBs persisted at the *YCR048w* hotspot (Figure 6, A and B). Progression in the absence of repair is a hallmark of defects in the meiotic recombination checkpoint. Therefore Mek1 kinase activity and Rec8 work together to promote a robust checkpoint response to unrepaired DSBs.

## DISCUSSION

**Regulation of meiotic intersister DSB repair occurs at the level of sister chromatids:** An important question is whether suppression of intersister DSB repair during meiosis is a locally regulated process occurring between sister chromatids as we have proposed (NIU *et al.* 2007) or whether the presence of homologous chromosomes somehow acts to channel recombination events away from sister chromatids. To distinguish between these possibilities we exploited the ability of budding yeast to undergo haploid meiosis, thereby creating a situation where the only templates available for repair are sister chromatids. Four different hotspots on three different chromosomes were examined and two completely independent approaches to inducing haploid meiosis were used. Therefore it is likely that our results reflect general properties of meiotic haploid chromosomes. We found that *dmc1*Δ haploids accumulate hyperresected DSBs, similar to *dmc1*Δ diploids, and that these breaks go away in the absence of Mek1. Therefore, Mek1 can inhibit Rad51-mediated strand invasion in the absence of homologous chromosomes, indicating that the mechanism of suppression is specific to sister chromatids.

In vegetative cells, a DSB on one chromosome results in the generation of replication-independent cohesion throughout the genome, indicating that DSBs can have global effects within a cell (STROM *et al.* 2007; UNAL *et al.* 2007). We exploited the haploid meiosis system to determine whether the presence of breaks on unpaired chromosomes affects DSB repair between homologs. Interhomolog recombination was observed between disomic chromosomes in cells where breaks on haploid chromosomes were not repaired, indicating that DSB repair is not coordinated between different pairs of homologous chromosomes. It should be noted, however, that interhomolog recombination was delayed and less efficient on the disomic chromosomes compared to the same homologous pair in a diploid. This may be because failure to repair breaks on haploid chromo-

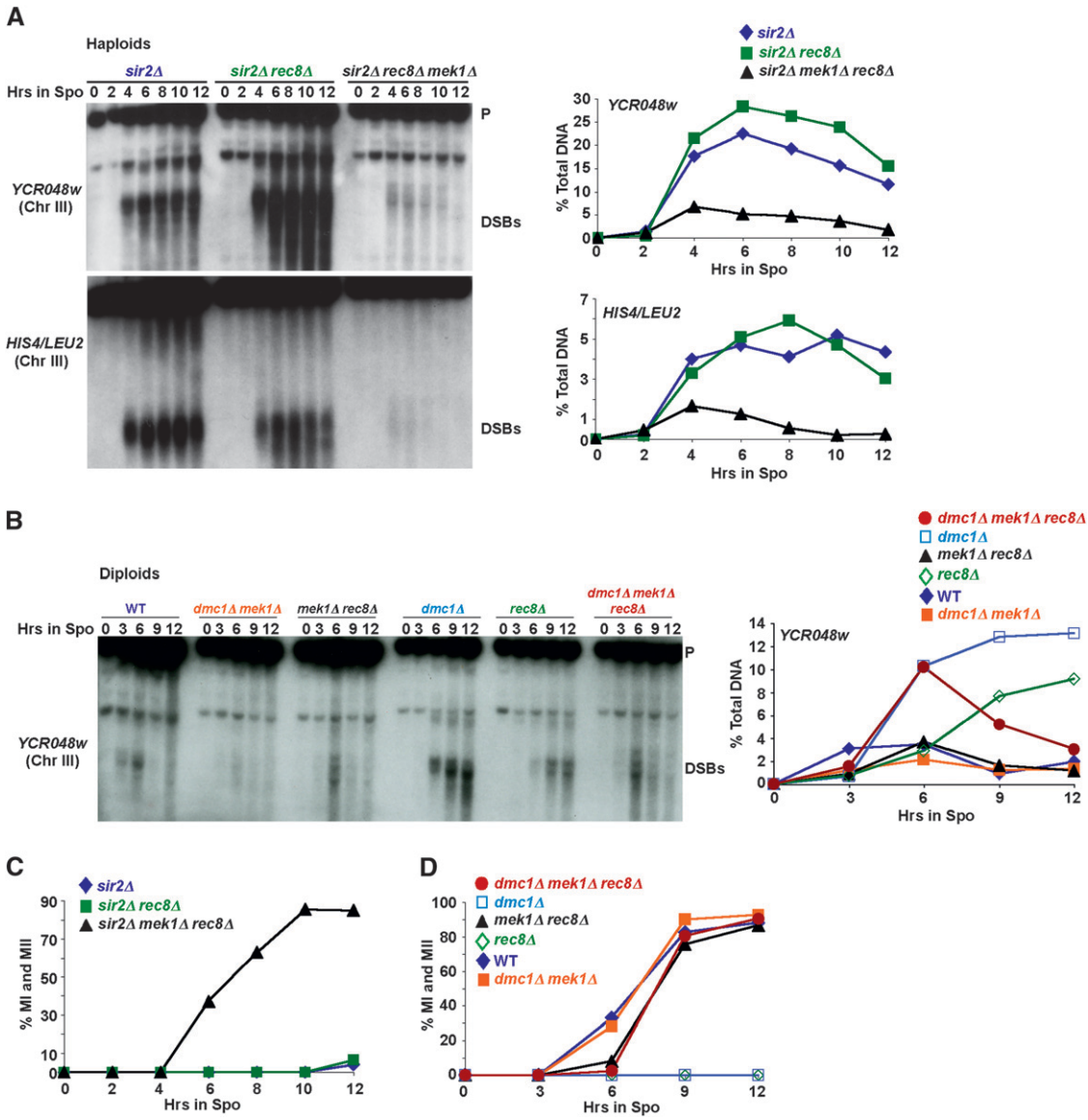


FIGURE 5.—DSB repair in various *rec8* strains. (A) Haploid strains: DSB repair examined at two different hotspots on chromosome III in *sir2*Δ (NHY1215 sir2), *sir2*Δ *rec8*Δ (NHY1215 sir2 rec8), and *sir2*Δ *rec8*Δ *mek1*Δ (NHY1215 sir2 rec8 mek1). (B) Diploid strains: DSB repair at the *YCR048w* hotspot in wild type (NH144), *mek1*Δ *rec8*Δ (NH751), *rec8*Δ (NH746), *dmc1*Δ (NH 748), *dmc1*Δ *mek1*Δ (NH749), and *dmc1*Δ *mek1*Δ *rec8*Δ (NH753). Graphs indicate quantitation of the DSB bands. (C) Meiotic progression in haploids from the time courses shown in A. (D) Meiotic progression in diploids from the time courses shown in B.

somes results in the accumulation of single-stranded DNA, thereby titrating out the recombination proteins that are available for repair. That recombination proteins are limiting in meiotic cells has previously been shown by JOHNSON *et al.* (2007).

**Rec8 cohesin complexes are not required for suppressing meiotic intersister DSB repair:** Given that Mek1 suppression of intersister DSB repair is specific to sister chromatids, a reasonable hypothesis is that the substrate(s) of Mek1 responsible for this suppression is associated with sister chromatids. One potential target is the multisubunit cohesin complex that holds sister chromatids together after DNA replication (ONN *et al.* 2008). In mitotic cells, DSBs promote the recruitment of Mcd1-containing cohesin complexes to break sites and the replication-independent establishment of cohesion throughout the genome (STROM *et al.* 2004, 2007; UNAL *et al.* 2004, 2007). DSB-dependent cohesion facilitates, but is not essential for, Rad51-mediated repair of DSBs using sister chromatids as templates. When *REC8* is ectopically expressed in mitotic cells in place of Mcd1, Rec8 does not localize to breaks, suggesting this is a property specific to Mcd1 (HEIDINGER-PAULI *et al.* 2008). During meiosis, however, *rec8*Δ diploids exhibit unrepaired DSBs, raising the possibility that Rec8 cohesin complexes might be required for intersister recombination (BRAR *et al.* 2009; KLEIN *et al.* 1999; KUGOU *et al.* 2009). Our work shows, however, that when suppression

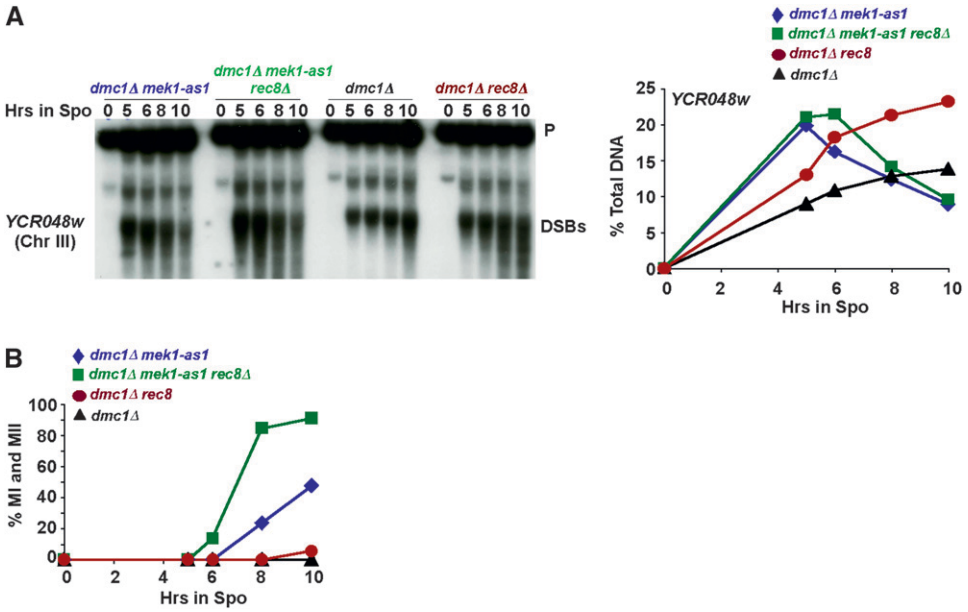


FIGURE 6.—Meiotic DSB repair and progression in *dmc1*Δ *mek1-as1* and *dmc1*Δ *mek1-as1 rec8*Δ diploids. Time courses were performed with *dmc1*Δ *mek1-as1* (NH749::pJR2), *dmc1*Δ *mek1-as1 rec8*Δ (NH 753::pJR2), *dmc1*Δ (NH748::pRS306), and *dmc1A rec8*Δ (NH752::pRS306). (A) DSBs were analyzed at the *YCR048w* hotspot. Graphs indicate quantitation of DSBs. (B) Meiotic progression from the time courses shown in A.

of intersister repair is relieved by deletion of *MEK1* in both haploids and diploids, *rec8*Δ DSBs are repaired. Therefore, *REC8* is not necessary for intersister DSB repair and instead specifically promotes interhomolog recombination.

**Rec8 cohesin complexes work with Mek1 in the meiotic recombination checkpoint:** Inhibition of Mek1 kinase activity in *dmc1*Δ strains allows meiotic progression because the signal to the meiotic recombination checkpoint—unrepaired DSBs—is removed by repairing the DSBs using sister chromatids as templates (NIU *et al.* 2005). In mutants that prevent processing of the breaks and their subsequent repair, eliminating Mek1 activity allows meiotic progression, indicating that Mek1 is required for the meiotic recombination checkpoint (XU *et al.* 1997). We found that combining a slightly less active version of *MEK1*, *mek1-as*, with a deletion of *REC8* eliminated the meiotic recombination checkpoint, whereas checkpoint activity was observed in the single mutant diploids. We propose that the effect of *rec8*Δ on the checkpoint is indirect. Genome-wide studies have shown that the distribution of Spo11 on chromosomes is altered in *rec8*Δ mutants, such that fewer breaks occur on chromosomes such as I, V, and VI (KUGOU *et al.* 2009). In contrast, little to no reduction in Spo11 localization or DSB formation was observed on chromosome III. Our model is that triggering the meiotic recombination checkpoint requires a threshold number of DSBs. Although the number of breaks generated in *rec8*Δ is reduced relative to wild type, this number is still above the threshold necessary for the checkpoint as cells arrest in meiotic prophase. Some DSB repair and meiotic progression were observed in the *mek1-as dmc1*Δ diploid used for these experiments, in contrast to *MEK1 dmc1*Δ, indicating that Mek1 activity is reduced by the analog-sensitive mutation. We propose that the weakened kinase activity of Mek1-as raises the threshold of DSBs required to trigger the checkpoint above the number formed in the *rec8*Δ, thereby preventing the checkpoint from detecting unrepaired breaks.

**Rad51 and Dmc1 recombinase activities are used differentially for sister chromatid and interhomolog DSB repair:** An unresolved issue in meiotic recombination is the roles that the different recombinases, Rad51 and Dmc1, play. Although several studies have indicated that Rad51 and Dmc1 are primarily involved in intersister and interhomolog recombination, respectively, *rad51*Δ mutants exhibit defects in both interhomolog joint molecule and crossover formation, suggesting that there may be overlapping functions as well (SHERIDAN and BISHOP 2006; HUNTER 2007). However, interpretation of the *rad51*Δ mutant is complicated by the fact that Rad51 is required for efficient loading of Dmc1 onto resected DSB ends (BISHOP 1994; SHINOHARA *et al.* 1997a). Therefore the interhomolog recombination defects of *rad51*Δ could be due in part to an indirect effect from a paucity of Dmc1.

Our studies suggest that the requirements for the recombinase activities of Rad51 and Dmc1 are distinct during meiosis. Wild-type haploid strains exhibit a delay or lack of DSB repair between sister chromatids (this work) (DE MASSY *et al.* 1994). Similar to the *dmc1*Δ diploids, this block to intersister repair is dependent upon *MEK1*, indicating that Dmc1, like Rad51, is constrained in haploid cells from invading sister chromatids by Mek1. However, our work shows that suppression of Dmc1-mediated repair between sister chromatids in haploids is indirect and dependent upon Rad51. This is consistent with a lack of homolog bias observed in diploids containing Dmc1-only filaments (SCHWACHA and KLECKNER 1997; LAO *et al.* 2008).

These results are consistent with previous studies suggesting that the Rad51 protein plays a structural role in proper assembly of Dmc1 onto filaments (Schwacha and Kleckner 1997; Hunter and Kleckner 2001; Sheridan and Bishop 2006; Lao *et al.* 2008). Filaments active for interhomolog recombination that contain only Rad51 can be generated by overexpressing *RAD51* or *RAD54*, deleting *HED1*, or preventing phosphorylation of *RAD54* in *dmc1*Δ strains (Bishop *et al.* 1999; Tsubouchi and Roeder 2003, 2006; Niu *et al.* 2009). In these cases, inactivation of Mek1 leads to repair of sister chromatids and dead spores. Therefore, the absence of Dmc1 has no effect on Mek1's ability to suppress Rad51 strand invasion of sister chromatids.

Our data support the proposal that in wild-type cells, Rad51's function in interhomolog recombination is to load Dmc1 onto breaks in a way that directs the filament toward homologous chromosomes instead of sister chromatids. How this actually works is unclear. One intriguing idea is that Rad51 confers different structural properties to the filament compared to Dmc1, but analysis of the biophysical properties of Rad51 and Dmc1 filaments formed *in vitro* revealed no obvious differences (Sheridan and Bishop 2006; Sheridan *et al.* 2008). After Dmc1 is loaded, Rad51 recombinase activity is shut down by Hed1 and Rad54 phosphorylation so that interhomolog recombination is then mediated exclusively by Dmc1. This situation allows Mek1 to act as a switch that controls when intersister DSB repair will occur. Inactivation of Mek1 allows Rad51/Rad54 complex formation and strand invasion of sister chromatids, perhaps to repair any remaining DSBs. The idea that Rad51/Rad54 may be used exclusively for sister recombination is supported by the fact that *rad54*Δ mutants exhibit wild-type levels of interhomolog recombination but still display reductions in sporulation and spore viability (Shinohara *et al.* 1997b; Schmuckli-Maurer and Heyer 2000).

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