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The Budding Yeast Mei5 and Sae3 Proteins Act Together With Dmc1 During Meiotic Recombination

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ABSTRACT

Here we provide evidence that the Mei5 and Sae3 proteins of budding yeast act together with Dmc1, a meiosis-specific, RecA-like recombinase. The *mei5* and *sae3* mutations reduce sporulation, spore viability, and crossing over to the same extent as *dmc1*. In all three mutants, these defects are largely suppressed by overproduction of Rad51. In addition, *mei5* and *sae3*, like *dmc1*, suppress the cell-cycle arrest phenotype of the *hop2* mutant. The Mei5, Sae3, and Dmc1 proteins colocalize to foci on meiotic chromosomes, and their localization is mutually dependent. The localization of Rad51 to chromosomes is not affected in either *mei5* or *sae3*. Taken together, these observations suggest that the Mei5 and Sae3 proteins are accessory factors specific to Dmc1. We speculate that Mei5 and Sae3 are necessary for efficient formation of Dmc1-containing nucleoprotein filaments *in vivo*.

MEIOSIS is a special type of cell cycle that produces haploid gametes from diploid parental cells. Unique to meiosis is the reductional division in which homologous chromosomes segregate to opposite poles. During the prophase that precedes this division, homologs pair with each other and undergo high levels of genetic recombination. Recombination plays at least two important roles in segregating chromosomes. First, it ensures that each chromosome finds its homolog. Second, a fraction of meiotic recombination events leads to reciprocal exchange (crossing over), which establishes physical connections between homologs. These connections, called chiasmata, ensure the proper alignment of chromosomes on the spindle apparatus at meiosis I.

The molecular mechanism of meiotic recombination has been well characterized in budding yeast. Meiotic recombination starts with double-strand breaks (DSBs) catalyzed by the Spo11 protein (Keeney 2001). Strands with 5′-termini at DSB ends are selectively degraded to leave single-strands with 3′-ends (Sun *et al.* 1991; Bishop *et al.* 1992), which are thought to be used for homology searching by strand-exchange proteins. The single-stranded DNA tails invade homologous sequences in nonsister chromatids to form single-end invasion intermediates, which may then be further processed to form double-Holliday junctions (Schwacha and Kleckner 1994; Hunter and Kleckner 2001).

During vegetative growth in budding yeast, gene products belonging to the *RAD52* epistasis group are involved in DSB repair through homologous recombination (Paques and Haber 1999; Symington 2002; Sung *et al.* 2003). Among them, Rad51, a homolog of the bacterial RecA protein, plays a major role in homologous DNA pairing and strand exchange. Rad51 functions in recombination by assembling as highly ordered oligomers on single-stranded DNA (Ogawa *et al.* 1993; Symington 2002). Formation of this structure, called a presynaptic filament, requires a group of accessory proteins, including Rad52 and the Rad55/57 heterodimer (Sung 1997a,b; New *et al.* 1998).

Rad51, Rad52, Rad55, and Rad57 also play essential roles in meiotic recombination (Borts *et al.* 1986; Shinohara *et al.* 1992; Schwacha and Kleckner 1997). In the absence of these proteins, DSBs are not efficiently converted to joint molecules. In wild type, formation of Rad51 presynaptic filaments is cytologically detectable at multiple distinct sites on chromatin, in response to DSB formation (Bishop 1994). Consistent with biochemical data, Rad52, Rad55, and Rad57 are necessary for foci formation by Rad51 during meiosis (Gasior *et al.* 1998).

Dmc1 is a meiosis-specific homolog of bacterial RecA that functions specifically in meiotic recombination (Bishop *et al.* 1992). In the absence of Dmc1, unrepaired DSBs accumulate with hyperresected ends (Bishop *et al.* 1992). *In vitro*, the Dmc1 protein promotes renaturation of complementary single-stranded DNA and assimilation of homologous single-stranded DNA into duplex DNA (Hong *et al.* 2001). Cytological studies have shown that Dmc1 forms many foci on chromosomes during meiosis, substantially overlapping with Rad51 foci (Bishop 1994).

Although both Rad51 and Dmc1 play important roles

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TABLE 1

Yeast strains

Strain	Genotype
S3246	*MAT***a** *his4-260 leu2-3,112 thr1-4 ura3-1 trp1-289 ade2-1* *MAT*α *his4-260 leu2-3,112 thr1-4 ura3-1 trp1-289 ade2-1*
TBR310	S3246 but homozygous *hop2::ADE2*
TBR737[a]	*MAT***a** *his4-Hpa leu2-3,112* *ARG4 thr1-4 CUP1 ura3-1 trp1-289 ade2-1 lys2* *MAT*α *his4-260 leu2::arg4-8-CUP1 CEN3::TRP1 arg4*Δ *thr1-4 cup1*Δ *ura3-1 trp1-289 ade2-1 lys2*
TBR751	S3246 but homozygous *mei5::KAN*
TBR821	S3246 but homozygous *MEI5-myc::KAN*
TBR860	S3246 but homozygous *hop2::ADE2 mei5::KAN*
TBR869	S3246 but homozygous *dmc1::KAN*
TBR885	S3246 but homozygous *hop2::ADE2 dmc1::KAN*
TBR1028[a]	TBR737 but homozygous *dmc1::KAN*
TBR1039	S3246 but homozygous *sae3::URA3*
TBR1044	S3246 but homozygous *hop2::ADE2 sae3::URA3*
TBR1053	S3246 but homozygous *dmc1::KAN mei5::KAN sae3::URA3*
TBR1063[a]	TBR737 but homozygous *sae3::KAN*
TBR1084	S3246 but homozygous *sae3::KAN*
TBR1087	S3246 but homozygous *hop2::ADE2 dmc1::KAN mei5::KAN sae3::URA3*
TBR1089[a]	TBR737 but homozygous *mei5::KAN*
TBR1098	S3246 but homozygous *spo11::ADE2 MEI5-myc::KAN*
TBR1099	S3246 but homozygous *hop2::ADE2 MEI5-myc::KAN*
TBR1136	S3246 but homozygous *dmc1::URA3 MEI5-myc::KAN*
TBR1247	S3246 but homozygous *sae3::URA3 MEI5-myc::KAN*
TBR1744	S3246 but homozygous *SAE3-myc::KAN*
TBR1772	S3246 but homozygous *spo11::ADE2 SAE3-myc::KAN*
TBR1773	S3246 but homozygous *hop2::ADE2 SAE3-myc::KAN*
TBR1774	S3246 but homozygous *dmc1::URA3 SAE3-myc::KAN*
TBR1775	S3246 but homozygous *mei5::KAN SAE3-myc::KAN*
TBR1797[a]	TBR737 but homozygous *dmc1::URA3 sae3::KAN*
TBR1798[a]	TBR737 but homozygous *dmc1::URA3 mei5::KAN*

[a] These diploid strains consist of a *MAT***a** strain isogenic with BR1919-8B and a *MAT*α strain congenic with BR1919-8B. The *MAT*α-bearing chromosomes in these strains are circular.

in meiotic recombination, a number of observations suggest that Rad51 plays a more critical role than Dmc1. First, localization of Rad51 to chromosomes is not affected in the absence of Dmc1, whereas localization of Dmc1 is severely reduced in the absence of Rad51 (Bishop 1994). Second, in a certain strain background, the *dmc1* mutant forms some viable spores (~20%), but essentially all spores are inviable in the *rad51* mutant (Rockmill *et al.* 1995b). Third, overproduction of Rad51 suppresses the *dmc1* defects in crossing over and viable spore production (Tsubouchi and Roeder 2003).

A recent study suggests that the early steps in meiotic recombination can proceed through two different pathways. One pathway relies on Rad51, but not Dmc1 (referred to as the Rad51-only pathway); the other utilizes both Dmc1 and Rad51 (referred to as the Dmc1-dependent pathway; Tsubouchi and Roeder 2003). Interestingly, the Dmc1-dependent pathway requires at least two proteins that are not involved in the Rad51-only pathway. These are Hop2 and Mnd1, two meiosis-specific proteins that interact with each other (Leu *et al.* 1998; Rabitsch *et al.* 2001; Gerton and DeRisi 2002; Tsubouchi and Roeder 2002). Epistasis analysis indicates that the Hop2/Mnd1 complex functions downstream of Dmc1 and is required for accurate homology searching in the Dmc1-dependent pathway (Tsubouchi and Roeder 2003). Previous studies have shown that the *dmc1* null mutant defect in meiotic cell-cycle progression varies in different yeast strain backgrounds. In an SK1 strain background, the *dmc1* mutant arrests in meiotic prophase (Bishop *et al.* 1992); however, in a BR2495 strain background, the *dmc1* mutant undergoes meiotic nuclear division after a delay (Rockmill *et al.* 1995b). This difference between strains could be due to different levels of Rad51 protein and/or differences in the relative activity of the Rad51-only pathway of meiotic recombination (Tsubouchi and Roeder 2003).

In the same way that Rad51 requires Rad52, Rad55, and Rad57 for efficient strand exchange, Dmc1 might require accessory factors. Since Dmc1 is produced only in meiotic cells, its accessory factors might also be meiosis specific. Two candidate genes that might encode

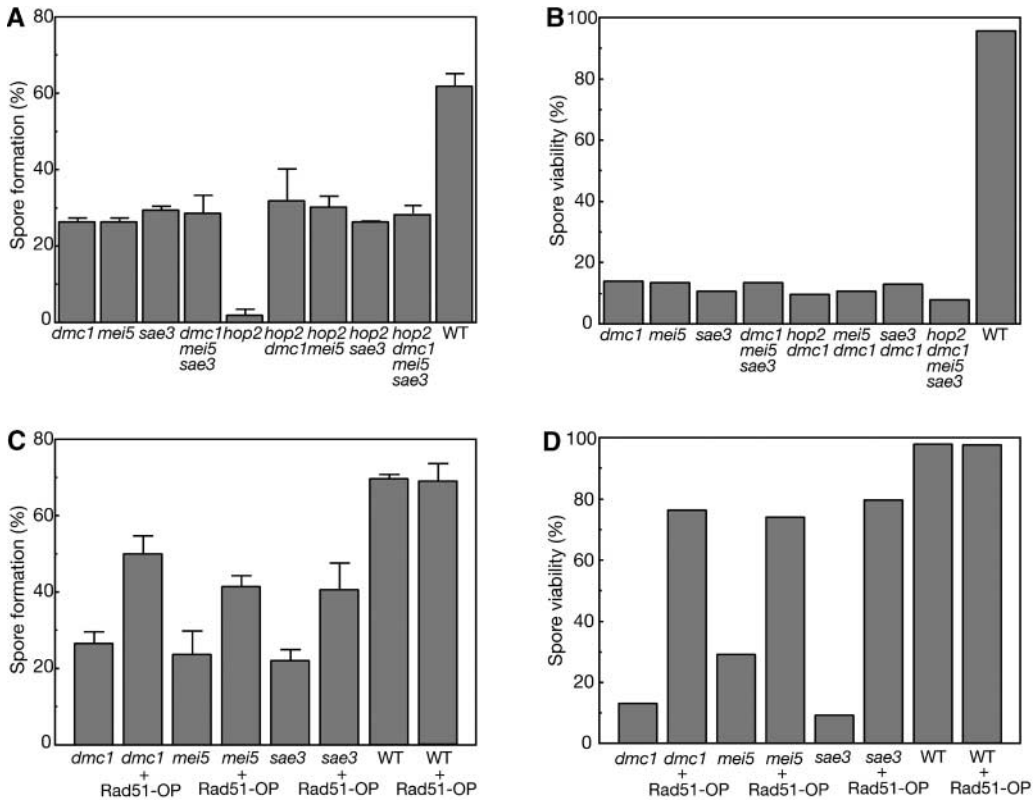

FIGURE 1.—Sporulation and spore viability in *mei5*, *sae3*, and *dmc1* strains carrying a *hop2* mutation or overproducing Rad51. (A and C) Cells were sporulated at 30° for 2 (A) or 3 (C) days; spore formation was measured as described in MATERIALS AND METHODS. Error bars represent standard deviations. (B and D) To measure spore viability, 44 tetrads were dissected for each strain. Strains analyzed in A and B are wild type (WT, S3246), *dmc1* (TBR869), *hop2* (TBR310), *mei5* (TBR751), *sae3* (TBR1039), *hop2 dmc1* (TBR885), *hop2 mei5* (TBR860), *hop2 sae3* (TBR1044), *dmc1 mei5 sae3* (TBR1053), and *hop2 dmc1 mei5 sae3* (TBR1087). Strains analyzed in C and D carry the multicopy vector YEpFAT4 containing either no insert or *RAD51*; only those carrying a vector with *RAD51* are indicated as + Rad51-OP. OP, overproduction. Plasmid-bearing strains were derived from wild type (WT, S3246), *dmc1* (TBR869), *mei5* (TBR751), and *sae3* (TBR1084).

Dmc1 accessory factors are *MEI5* and *SAE3*. In the SK1 strain background, the *mei5* mutant fails to undergo efficient nuclear division despite an apparently normal premeiotic S phase (RABITSCH *et al.* 2001). This phenotype is one of the hallmarks of mutants defective in the Dmc1-dependent recombination pathway (BISHOP *et al.* 1992). The *sae3* mutant also arrests at meiotic prophase in SK1 strains. Meiotic DSBs become hyperresected, and the production of mature recombinants is reduced to ~10% of the wild-type level (MCKEE and KLECKNER 1997). Recently, the *SAE3* gene was proposed to have an intron and to encode a protein homologous to the fission yeast Swi5 protein (AKAMATSU *et al.* 2003). Here, we present genetic and cytological evidence indicating that the Mei5 and Sae3 proteins act in the Dmc1-dependent pathway of meiotic recombination.

MATERIALS AND METHODS

Yeast strains and plasmids: Yeast strains used in this study are summarized in Table 1. Strains are isogenic with the haploid strain BR1919-8B (ROCKMILL and ROEDER 1990), unless otherwise stated. Isogenic derivatives of S3246 were constructed by mating appropriate haploids; isogenic haploids were generated by transformation and/or by genetic crosses. BR1919-8B strains carrying *sae3::URA3* were obtained from J. Novak (Yale University, New Haven, CT).

The following constructs were described previously: YEpFAT4-*RAD51* (TSUBOUCHI and ROEDER 2003) and plasmids for introducing *hop2::ADE2* (TSUBOUCHI and ROEDER 2002) and *dmc1::URA3* (BISHOP *et al.* 1992) disruptions. *dmc1::KAN*, *mei5::KAN*, and *sae3::KAN* were engineered by PCR-mediated gene disruption (LONGTINE *et al.* 1998). The *kanMX* cassette was used to replace the entire open reading frame (ORF) of *DMC1* and *MEI5* and the previously annotated ORF for *SAE3* (MCKEE and KLECKNER 1997). Sequences encoding 13 copies of the myc epitope were integrated at the 3′-end of the *MEI5* gene and at the 3′-end of the newly annotated *SAE3* ORF by the PCR-mediated method of LONGTINE *et al.* (1998).

Cytology: Meiotic chromosomes were surface spread, and immunostaining was carried out as described previously (SYM *et al.* 1993; CHUA and ROEDER 1998). Rabbit and mouse anti-Red1 antibodies were used at 1:500 dilution (SMITH and ROEDER 1997). Rabbit anti-Rad51 and anti-Dmc1 antibodies were used at 1:500 dilution (SHINOHARA *et al.* 1992; BISHOP 1994). Rabbit anti-Zip1 antibodies were used at 1:100 dilution (SYM *et al.* 1993). Mouse anti-myc antibody was used at 1:100 dilution (Covance).

RT-PCR: RT-PCR was done as described previously (TSUBOUCHI and ROEDER 2002). RNA was reverse transcribed using the following primer: TTAGTCCTTCATTGAATAACCGAT. The resulting cDNA was amplified using the above primer and the following primer: ATGAACTATTTGGAAACACAGTTA.

Measuring sporulation: From single colonies, patches were made on YPD plates (or on selection plates when necessary) and incubated at 30° overnight. The plates were replica plated onto sporulation medium and incubated at 30° for 2 or 3 days. For each strain, spore formation was measured in three

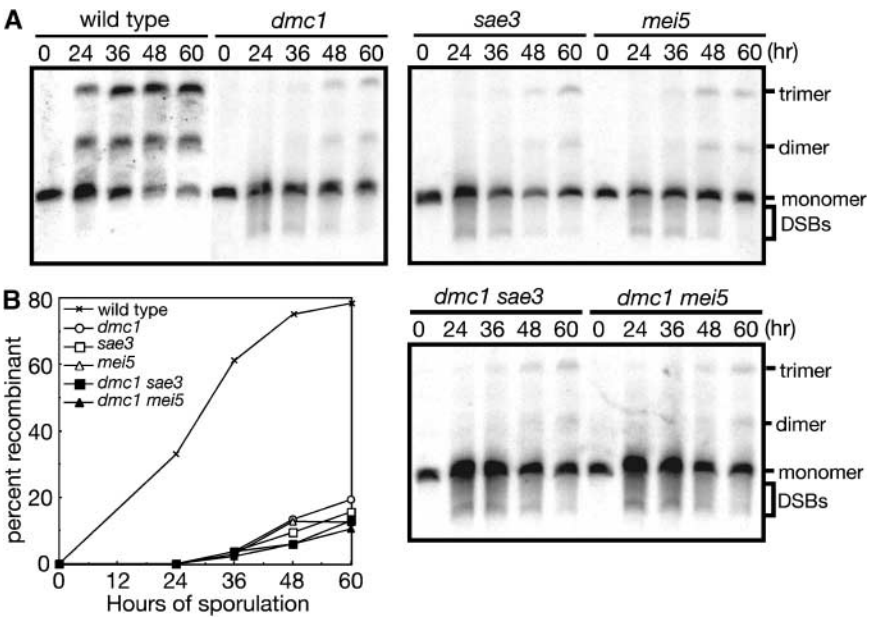

FIGURE 2.—Crossing over is reduced in *dmc1*, *mei5*, and *sae3* mutants. (A) Diploids containing one circular and one linear copy of chromosome III were introduced into sporulation medium and samples were harvested at the time points indicated. Genomic DNA was subjected to pulsed-field gel electrophoresis followed by Southern blot analysis, hybridizing with a probe containing the *THR4* gene on chromosome III (CHUA and ROEDER 1998; AGARWAL and ROEDER 2000). The positions of linear monomers, dimers, and trimers and of molecules that have sustained one or more DSBs are indicated to the right of the gel. (B) Quantitative analysis of physical recombinants. To calculate the percentage of DNA in crossover products (percentage of recombinants), the sum of signals for dimer and trimer molecules was divided by the sum of signals for monomers, dimers, and trimers (plus DSBs if applicable) for each lane. Strains analyzed are wild type (WT, TBR737), *dmc1* (TBR1028), *sae3* (TBR1063), *mei5* (TBR1089), *dmc1 sae3* (TBR1797), and *dmc1 mei5* (TBR1798).

independent experiments, with at least 300 cells scored in each experiment.

Statistics: To compare the colocalization of Mei5 (or Sae3) with Dmc1 *vs.* Rad51, contingency chi-square tests were performed. For example, for chromosome spreads stained for both Mei5 and Dmc1, Mei5 foci were divided into two groups: those colocalizing with Dmc1 and those not colocalizing; similarly, the numbers of Mei5 foci colocalizing or not colocalizaing with Rad51 were determined. Using these two sets of numbers, a chi-square value was calculated, and the corresponding probability (*P*-value) was obtained. Using the same method, the fraction of Dmc1 foci containing Mei5 was compared to the fraction of Rad51 foci containing Mei5. The same methods were used to assess overlap between Sae3 and Dmc1/Rad51.

RESULTS

Genetic analysis places *MEI5* and *SAE3* in the same recombination pathway as *DMC1*: To further characterize the *mei5* and *sae3* mutants, the *MEI5* and *SAE3* genes were deleted in the BR1919-8B strain background, in which the *dmc1* mutant sporulates with reduced efficiency (TSUBOUCHI and ROEDER 2003). Both the *mei5* mutant and the *sae3* mutant exhibit levels of sporulation and spore viability similar to *dmc1* (Figure 1, A and B). The *dmc1 mei5 sae3* triple mutant also shows the same levels of sporulation and spore viability as each single mutant (Figure 1, A and B), strongly suggesting that the affected gene products work in the same pathway.

The *hop2* mutant shows complete cell-cycle arrest in the BR1919-8B background (TSUBOUCHI and ROEDER 2002). The *dmc1 hop2* double mutant sporulates to the same extent as *dmc1*, indicating that *dmc1* is epistatic to *hop2* (Figure 1A; TSUBOUCHI and ROEDER 2003). The *mei5 hop2* and *sae3 hop2* double mutants show sporulation and spore viability similar to *dmc1 hop2* (Figure 1, A and B), indicating that *mei5* and *sae3* also suppress *hop2*. Furthermore, the *hop2 dmc1 mei5 sae3* quadruple mutant is indistinguishable from the double mutants (Figure 1, A and B). Thus, it is likely that Mei5, Sae3, and Dmc1 act in the same recombination pathway at a step upstream of Hop2/Mnd1.

Another phenotype characteristic of mutants defective in the Dmc1-dependent recombination pathway is that their defects in sporulation and viable spore production can be suppressed by overproduction of Rad51 (TSUBOUCHI and ROEDER 2003). Overproduction of Rad51 improves spore formation and viability in the *mei5* and *sae3* mutants to an extent similar to that seen in the *dmc1* mutant (Figure 1, C and D). This observation provides further evidence that Mei5 and Sae3 are involved in the Dmc1-dependent recombination pathway.

The *mei5*, *sae3*, and *dmc1* mutations reduce crossing over to the same extent: To determine the effect of *mei5* on the formation of mature recombinants and to compare *mei5* with *dmc1* and *sae3* (BISHOP *et al.* 1992; MCKEE and KLECKNER 1997), crossing over was assayed physically in diploid strains carrying one linear and one circular copy of chromosome III. A single crossover between one linear and one circular chromatid results in production of a linear dimer. A double crossover involving one linear chromatid and both chromatids of the circular chromosome generates a linear trimer. The linear monomers, dimers, and trimers can be separated by pulsed-field gel electrophoresis (circular chromosomes do not enter the gel). In wild type, after 60 hr of sporulation, ~80% of total DNA entering the gel is

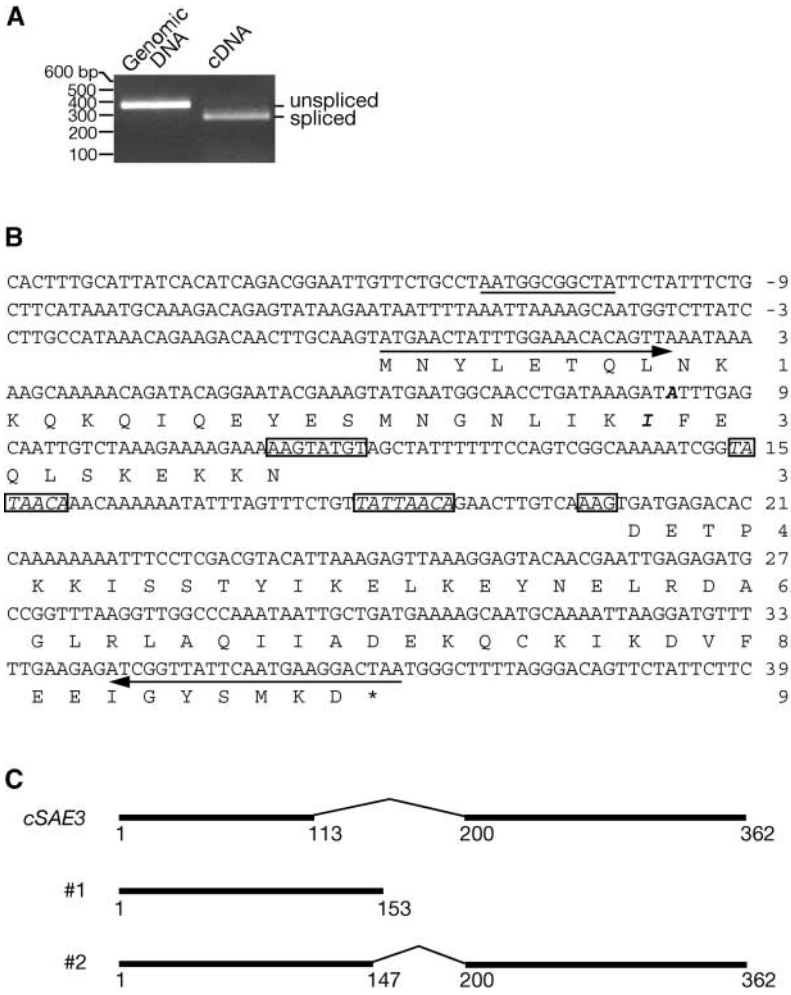

FIGURE 3.—The *SAE3* gene has an intron. (A) Genomic and cDNA derived from meiotic mRNA were amplified by PCR with primers and analyzed by agarose gel electrophoresis. (B) DNA sequence and encoded amino acid sequence of *SAE3*. Consensus splicing signals are boxed. Italicized boxed sequences indicate potential branchpoint sequences (see text). A URS1 consensus sequence is underlined. Primers used for PCR amplification are shown with arrows. Compared to the sequence of strain S288c published in the Saccharomyces Genome Database (http://www.yeastgenome.org), the SK1 strain from which *SAE3* cDNA was derived has a polymorphism at the 83rd nucleotide—A (italicized in boldface type) instead of G. The single nucleotide substitution changes the 28th amino acid from M to I (italicized in boldface type). Numbering starts at the first nucleotide of the first codon for the DNA sequence and the first amino acid for the amino acid sequence. (C) Structure of *SAE3* ORFs. The structure of the cDNA found in this study is shown on top. Structure 1 was proposed by McKEE and KLECKNER (1997). Structure 2 was proposed by AKAMATSU *et al.* (2003).

recombinant (Figure 2, A and B). In all three mutants, the band representing linear monomers is accompanied by a substantial amount of DNA migrating faster than the intact monomer (Figure 2, A and B). These fragments of lower molecular weight represent molecules resulting from double-strand breakage (ROCKMILL *et al.* 1995a), and their persistence indicates that the mutants are defective in DSB repair. Furthermore, the mutants produce a smaller amount of linear dimers and trimers compared to wild type, and these do not appear until much later than their wild-type counterparts (Figure 2, A and B). The timing and extent of crossing over are similar in the *mei5*, *sae3*, and *dmc1* mutants.

Consistent with the spore formation and viability data shown in Figure 1, the *dmc1 sae3* and *dmc1 mei5* double mutants show essentially the same phenotype as each single mutant with respect to the extent and kinetics of crossover formation (Figure 2, A and B). These data further support the conclusion that Dmc1, Mei5, and Sae3 act in the same pathway.

The *SAE3* gene contains an intron: Recently, the *SAE3* gene was proposed to have an intron and to encode a member of a family of proteins homologous to the fission yeast Swi5 protein (AKAMATSU *et al.* 2003). To confirm that *SAE3* pre-mRNA is spliced, mRNA from meiotic cells was reverse transcribed, and the resultant cDNA was amplified by PCR using primers flanking any potential introns. The DNA amplified from cDNA was found to be smaller than the DNA amplified from genomic DNA, indicating that the mRNA is indeed spliced (Figure 3A). The sequence of the cDNA revealed a new ORF for the *SAE3* gene (Figure 3, B and C), with a different 5′ splice junction from the one predicted previously (AKAMATSU *et al.* 2003). The intron contains a well-conserved 5′ splice site and a 3′ splice site that is used relatively infrequently (Figure 3B; RYMOND and ROSBASH 1992). However, no sequence matching the conserved branchpoint sequence (TACTAACA; RYMOND and ROSBASH 1992) is present. Instead, two closely related sequences are found (Figure 3B). In the first (TATAACA), the third C in the consensus sequence is missing. In the second (TATTAACA), the third C in the consensus sequence is replaced with T. In summary, the *SAE3* gene contains an intron 86 nucleotides in length and encodes a protein of 91 amino acids (Figure 3B).

The Mei5 and Sae3 proteins localize to chromosomes as foci in a *SPO11*-dependent manner: To determine the *in vivo* localization of Mei5 and Sae3, the proteins

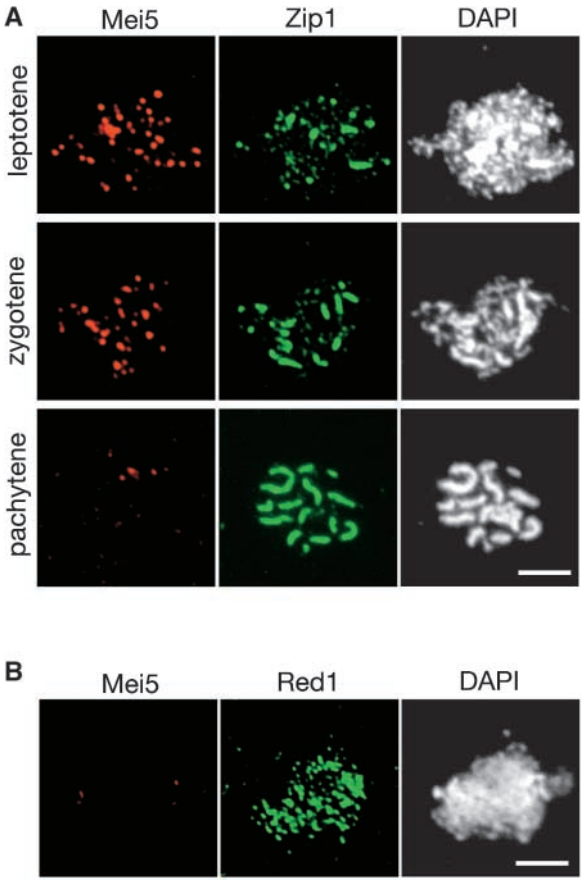

FIGURE 4.—Localization of Mei5 to meiotic chromosomes. (A) Meiotic chromosomes from wild-type cells producing myc-tagged Mei5 (TBR821) were stained with anti-myc and anti-Zip1 antibodies and with the DNA-binding dye 4′,6′-diamidino-2-phenylindole (DAPI). (B) Nuclei from *spo11* cells producing myc-tagged Mei5 (TBR1098) were stained with anti-myc and anti-Red1 antibodies and with DAPI. Bars, 4 μm.

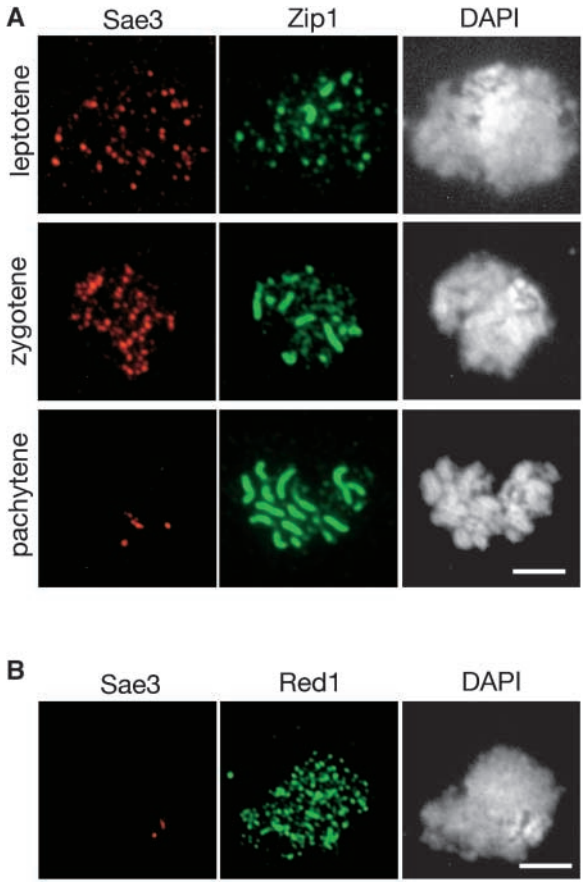

FIGURE 5.—Localization of Sae3 to meiotic chromosomes. (A) Meiotic chromosomes from wild-type cells producing myc-tagged Sae3 (TBR1744) were stained with anti-myc and anti-Zip1 antibodies and with DAPI. (B) Nuclei from *spo11* cells producing myc-tagged Mei5 (TBR1772) were stained with anti-myc and anti-Red1 antibodies and with DAPI. Bars, 4 μm.

were tagged with the myc epitope and immunolocalized in spread meiotic nuclei. Diploid strains in which both copies of the *MEI5* (or *SAE3*) gene have been replaced by *MEI5-myc* (or *SAE3-myc*) display wild-type levels of spore viability (data not shown), indicating that the tagged proteins are functional (data not shown). Cells at different stages of meiotic prophase were identified on the basis of the staining pattern of the Zip1 protein, which is a major component of the synaptonemal complex and thus serves as a marker for chromosome synapsis (SYM *et al.* 1993). The Mei5 and Sae3 proteins are present as numerous foci on chromosomes at leptotene (dotty Zip1 staining) and zygotene (dotty plus linear Zip1 staining), but these foci largely disappear by pachytene (linear Zip1; Figures 4A and 5A). This localization pattern is similar to that seen for Rad51 and Dmc1 (BISHOP 1994).

To test if Mei5 and Sae3 localization to chromosomes requires the initiation of meiotic recombination, these proteins were immunolocalized in the *spo11* mutant. To identify cells at prophase I, chromosomes were simultaneously stained with antibodies to the Red1 protein, which is a component of the cores of meiotic chromosomes. Unlike Zip1, Red1 localizes to chromosomes independently of meiotic recombination (SMITH and ROEDER 1997). Like Rad51 and Dmc1 (BISHOP 1994; GASIOR *et al.* 1998), little or no Mei5 or Sae3 is present on chromosomes in the *spo11* mutant (Figures 4B and 5B). The residual Mei5/Sae3 foci observed in *spo11* are at least five times less intense than those seen in wild type. These faint foci may reflect nonspecific sticking of the Mei5 and Sae3 proteins to chromosomes. Alternatively, Mei5 and Sae3 might localize weakly to chromosomes even in the absence of DSBs. Taken together, these results are consistent with direct roles for Mei5 and Sae3 in meiotic recombination.

Localization of the Mei5, Sae3, and Dmc1 proteins to chromosomes is mutually dependent: The kinetics of foci formation by Mei5, Sae3, Rad51, and Dmc1 are similar, raising the possibility that these proteins work together in the meiotic recombination pathway. How-

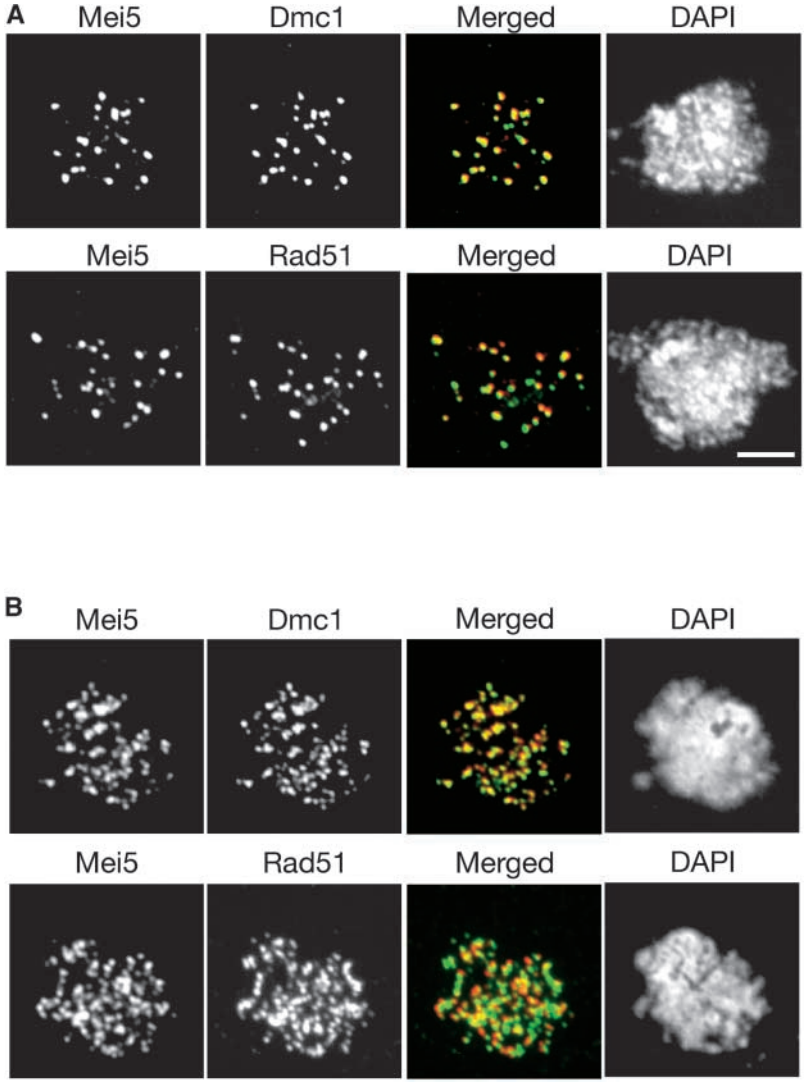

FIGURE 6.—Co-immunostaining of Mei5 with Rad51 or Dmc1. Meiotic chromosomes from (A) wild type producing myc-tagged Mei5 (TBR821) or (B) the *hop2* mutant producing myc-tagged Mei5 (TBR1099) were stained with anti-myc and either anti-Rad51 or anti-Dmc1 antibodies and with DAPI. Bar, 4 μm.

ever, previous studies showed that Rad51 and Dmc1 foci do not completely overlap (DRESSER *et al.* 1997; SHINOHARA *et al.* 2000). To examine if Mei5 and Sae3 associate with one or both of these RecA homologs, co-immunostaining of Dmc1 and either Mei5 or Sae3 and of Rad51 and either Mei5 or Sae3 was carried out.

Both Mei5 and Sae3 show extensive colocalization with both Dmc1 and Rad51 (Figures 6A and 7A). However, the extent of overlap is much greater for Dmc1 than for Rad51 (Table 2). This trend is most pronounced in the *hop2* mutant background, in which all four proteins accumulate on chromosomes (Figures 6B and 7B; Table 2).

The formation of Mei5 and Sae3 foci is largely abolished in the *dmc1* mutant (Figure 8A). Similarly, formation of Dmc1 and Sae3 foci is nearly eliminated in the *mei5* mutant (Figure 8B), and formation of Dmc1 and Mei5 foci is abolished in the *sae3* mutant (Figure 8C). Thus, localization of Mei5, Sae3, and Dmc1 to chromosomes is mutually dependent. In each case, some residual staining of low signal intensity is observed on chromosome spreads. This staining may reflect nonspecific background staining or a low level of localization for each protein in the absence of its partners.

Previous studies showed that the absence of Dmc1 does not affect the localization of Rad51 (BISHOP 1994). The localization of Rad51 was tested in the *dmc1*, *mei5*, and *sae3* mutants. As expected, Rad51 still forms foci in these mutants (Figure 9).

DISCUSSION

The Mei5 and Sae3 proteins work in the Dmc1-dependent recombination pathway: Here, we provide genetic and cytological evidence that the Mei5 and Sae3 proteins work at the same step as Dmc1 in the Dmc1-dependent meiotic recombination pathway. First, mutations in all three genes reduce sporulation and spore viability to the same extent. Second, like *dmc1*, *mei5* and *sae3* mutations suppress cell-cycle arrest in the *hop2* mutant.

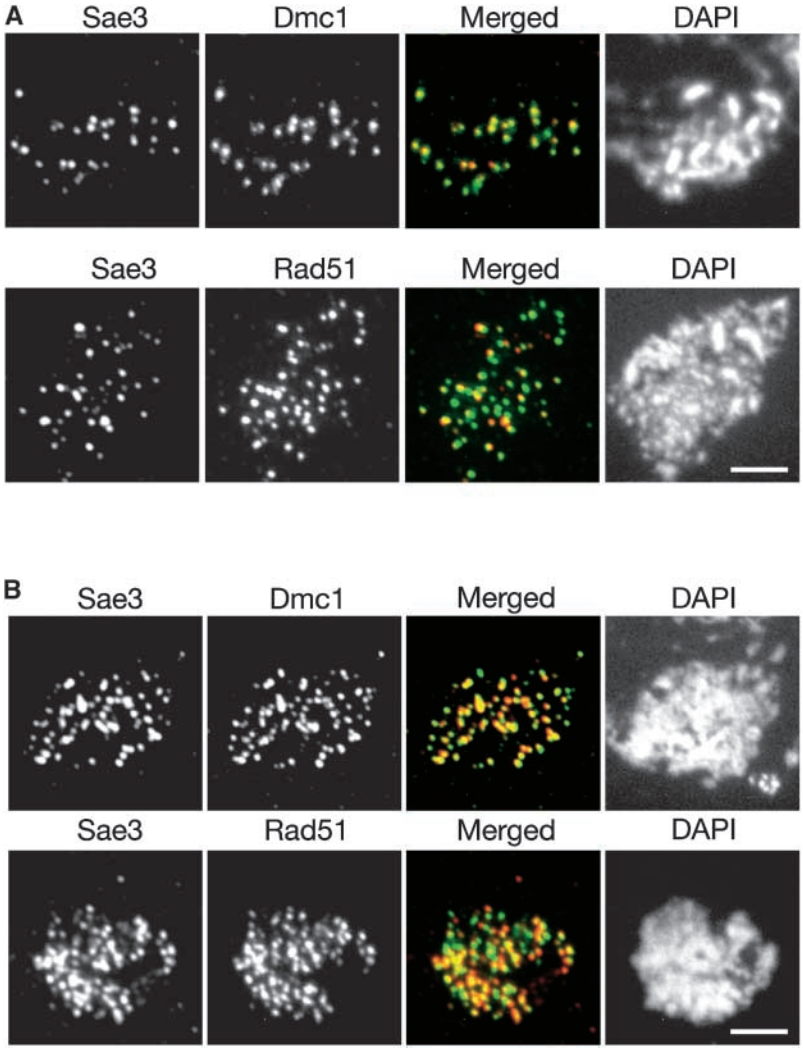

Figure 7.—Co-immunostaining of Sae3 with Rad51 or Dmc1. Meiotic chromosomes from (A) wild type producing myc-tagged Sae3 (TBR1744) or (B) the *hop2* mutant producing myc-tagged Sae3 (TBR1773) were stained with anti-myc and either anti-Rad51 or anti-Dmc1 antibodies and with DAPI. Bars, 4 μm.

Third, Rad51 overproduction suppresses the *mei5* and *sae3* defects in sporulation and spore viability, as is the case for *dmc1*. Fourth, physical analysis of recombination intermediates and products indicates that *mei5*, *sae3*, and *dmc1* reduce meiotic DSB repair and crossing over to a similar extent, and recombination is reduced to the same degree in the *dmc1 mei5* and *dmc1 sae3* double mutants. Fifth, the Mei5 and Sae3 proteins are found together with Dmc1 as many foci on chromosomes during prophase I, with the number increasing from leptotene through zytotene and then disappearing through pachytene. Furthermore, the localization of Mei5, Sae3, and Dmc1 to chromosomes is mutually dependent.

The Mei5 and Sae3 proteins may be accessory factors to Dmc1: It seems likely that Dmc1 is directly involved in homology searching and strand exchange in meiotic recombination for the following reasons. First, Dmc1 is a homolog of the bacterial RecA protein (Bishop *et al.* 1992). Second, the *dmc1* mutant is strongly defective in meiotic recombination. In the SK1 strain background, there is robust accumulation of hyperresected DSBs (Bishop *et al.* 1992). Furthermore, recombination intermediates such as single-end invasions and double Holliday junctions are completely absent in *dmc1* SK1 strains (Schwacha and Kleckner 1995; Hunter and Kleckner 2001).

Early studies of Dmc1 protein purified from yeast and human cells failed to detect the strong strand-exchange activity characteristic of Rad51 (Li *et al.* 1997; Masson *et al.* 1999; Hong *et al.* 2001). Furthermore, the human Dmc1 protein was originally reported to form stacked ring structures on DNA (Masson *et al.* 1999; Passy *et al.* 1999) instead of the helical nucleoprotein filaments reported for RecA and Rad51 (Dunn *et al.* 1982; Flory and Radding 1982; Ogawa *et al.* 1993). Recently, however, human Dmc1 was found to have strong strand-exchange activity *in vitro*, although this activity is highly sensitive to salt concentration and pH (Sehorn *et al.* 2004). Furthermore, under conditions conducive to strand exchange *in vitro*, human Dmc1 forms helical nucleoprotein filaments similar in appearance to those formed by Rad51 (Sehorn *et al.* 2004).

TABLE 2

Colocalization of foci

	Colocalization frequencies (%)									
	Wild type					*hop2*				
	$Dmc1^{Y}$	*n*	$Rad51^{Y}$	*n*	*P*-value	$Dmc1^{Y}$	*n*	$Rad51^{Y}$	*n*	*P*-value
$Mei5^{X}$	86.8	311	73.3	592	4.9×10^{-6}	97.9	1064	85.9	808	6.7×10^{-23}
	86.5	312	78.5	553	4.6×10^{-3}	97.3	1071	79.7	1064	1.2×10^{-35}
$Sae3^{X}$	95.3	465	85.3	517	3.4×10^{-7}	97.8	1002	93.7	1164	6.8×10^{-6}
	95.1	466	86.8	508	1.5×10^{-5}	97.5	1005	87.0	1257	5.8×10^{-19}

For each pair of proteins, there are two rows of numbers, with the first row indicating the percentage of foci of protein X (Mei5 or Sae3) that overlaps with foci of protein Y (Dmc1 or Rad51); the second row indicates the percentage of foci of protein Y that overlaps with protein X. *n* indicates the total number of foci of protein X (first row for each protein) or Y (second row for each protein) that were scored; the number of nuclei examined ranged from 8 to 15. The *P*-value indicates the probability that the frequency of overlap between Rad51 and Mei5 (or Sae3) is the same as the frequency of overlap between Dmc1 and Mei5 (or Sae3). The method used to calculate *P*-values is described in MATERIALS AND METHODS.

Like Rad51, Dmc1 may require special cofactors for maximal filament formation and strand-exchange activity *in vivo*. The Mei5 and Sae3 proteins are obvious candidates for these cofactors, since Dmc1 cannot localize to chromosomes in their absence. The following observations strongly suggest that Mei5 and Sae3 act in conjunction with Dmc1 as components of the recombination machinery. First, the Mei5 and Sae3 proteins localize to chromosomes as foci, and the timing of appearance and disappearance of these foci is consistent with the appearance and disappearance of DSBs (CAO *et al.* 1990; PADMORE *et al.* 1991). Second, localization of Mei5 and Sae3 to chromosomes is dependent on the initiation of meiotic recombination. Third, Mei5 and

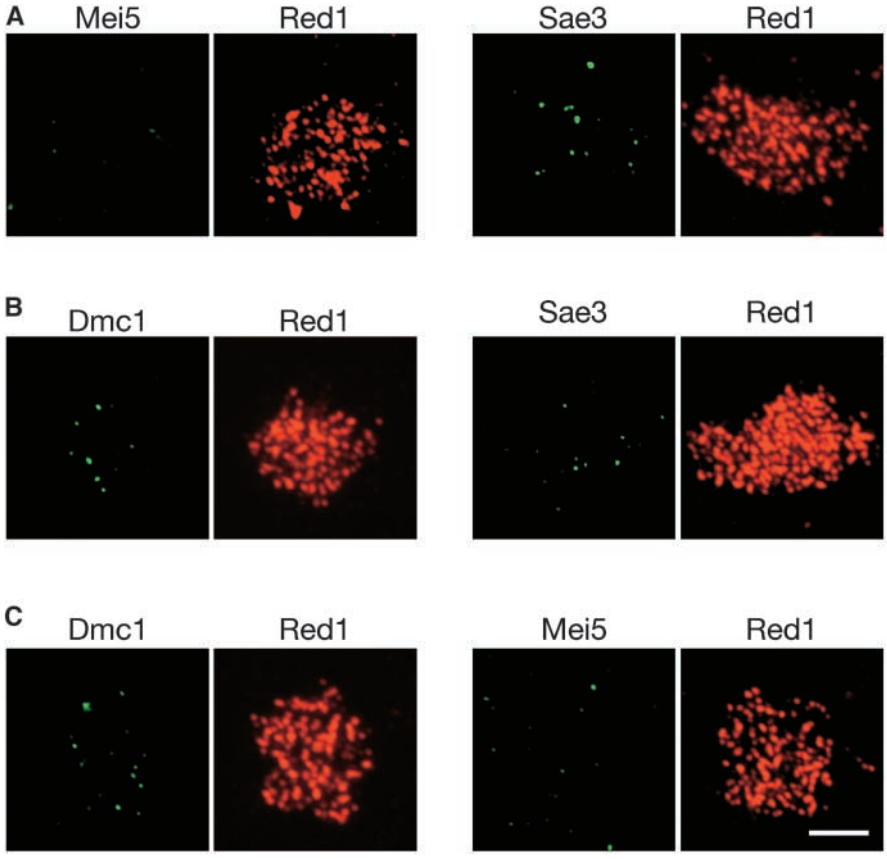

FIGURE 8.—Chromosomal localization of Mei5, Dmc1, and Sae3 is mutually dependent. (A) Nuclei from a *dmc1* mutant producing either myc-tagged Mei5 (TBR1136) or myc-tagged Sae3 (TBR1774) were stained with anti-myc and anti-Red1 antibodies. (B) Nuclei from a *mei5* mutant (TBR751) and a *mei5* mutant producing myc-tagged Sae3 (TBR1775) were stained with anti-Dmc1 or anti-myc and anti-Red1 antibodies. (C) Nuclei from the *sae3* mutant producing myc-tagged Mei5 (TBR1247) were stained with anti-Red1 and either anti-Dmc1 or anti-myc antibodies. Bar, 4 μm.

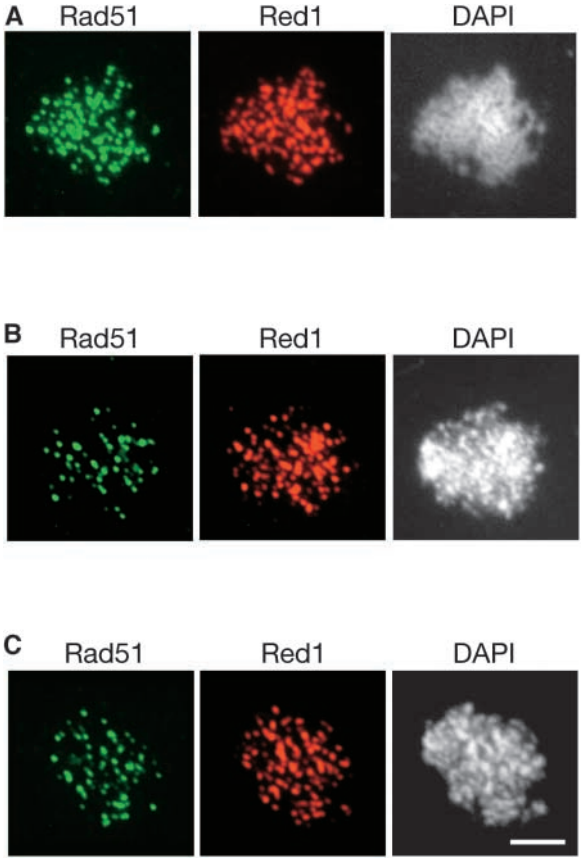

Figure 9.—Rad51 localizes to chromosomes in the *dmc1*, *mei5*, and *sae3* mutants. Meiotic chromosomes from (A) *dmc1* (TBR869), (B) *mei5* (TBR751), and (C) *sae3* (TBR1039) mutants were stained with anti-Rad51 and anti-Red1 antibodies and also with DAPI. Bar, 4 μm.

Sae3 foci significantly overlap with Dmc1 foci. Fourth, the localization of Mei5, Sae3, and Dmc1 to chromosomes is mutually dependent. We propose that the Mei5 and Sae3 proteins are necessary for efficient filament formation and/or strand exchange mediated by Dmc1.

Although both Mei5 and Sae3 are necessary for Dmc1 localization on chromosomes, they are not required for Rad51 localization, suggesting that they are factors specific for Dmc1. On the other hand, Rad51 is necessary for wild-type levels of localization of Dmc1, Mei5, and Sae3 to chromosomes (Bishop *et al.* 1992; our unpublished data). One possibility for this Rad51 dependency is that certain recombination intermediates created by Rad51 are necessary for efficient loading of the Dmc1, Sae3, and Mei5 proteins onto chromosomes. Alternatively, a protein-protein interaction between Rad51 and these proteins might be necessary for their chromosomal localization.

How might Mei5 and Sae3 promote Dmc1-mediated strand exchange? Rad51-mediated strand exchange occurs in a number of steps (Sung *et al.* 2003). First, Rad51 assembles on single-stranded DNA to form a helical filament, called the presynaptic filament. Next is the synaptic phase during which duplex DNA is incorporated into the filament and sampled for homology. Once homology is found, alignment between the duplex and the single strand becomes stabilized. The final step is strand exchange during which the complementary strand from the duplex is progressively taken up into the filament.

At what stage might Mei5 and Sae3 act, assuming that strand exchange by Rad51 and Dmc1 are mechanistically related? Our genetic results suggest that the Mei5 and Sae3 proteins, just like Dmc1, act upstream of the step that involves Hop2/Mnd1. The Mei5, Sae3, and Dmc1 proteins accumulate excessively in the *hop2* mutant. These phenotypes argue that Mei5 and Sae3 act together with Dmc1 before the strand-exchange step in the recombination reaction. The Hop2/Mnd1 complex is proposed to promote accurate homology searching in the Dmc1-dependent recombination pathway (Tsubouchi and Roeder 2003). In the absence of either Hop2 or Mnd1, interactions between nonhomologous sequences become inappropriately stabilized and nucleate synaptonemal complex formation. Taken together, these results suggest that Mei5 and Sae3 act either in the formation of presynaptic filaments or in the synaptic phase of homology searching.

A number of proteins are known to be involved in the assembly of presynaptic filaments involving Rad51, including RPA, Rad52, Rad54, Rad55, and Rad57 (Paques and Haber 1999; Symington 2002; Sung *et al.* 2003). RPA is a single-stranded DNA-binding protein complex that plays a role in removing secondary structure from single-stranded DNA. Interestingly, RPA competes with Rad51 for binding DNA; when single-stranded DNA is covered with RPA before Rad51, assembly of the Rad51 presynaptic filament is hindered (Sugiyama *et al.* 1997; Sung 1997b). Rad52, Rad54, and the Rad55-Rad57 complex promote nucleation of Rad51 by overcoming the inhibitory effect of RPA (Sung *et al.* 2003; Wolner *et al.* 2003). Rad54 is a nonessential component of the Rad51 filament; Rad54/Rad51 nucleoprotein filaments are more stable than filaments lacking Rad54. Rad54 may also be active in the synaptic phase where it has been postulated to endow the presynaptic filament with the ability to actively scan duplex DNA for homology (Petukhova *et al.* 1998, 2000).

It is likely that at least some Rad51 accessory factors are also involved in Dmc1-mediated strand exchange *in vivo*. Indeed, strand exchange *in vitro* mediated by the human Dmc1 protein absolutely requires RPA and is enhanced in the presence of Rad54B (Sehorn *et al.* 2004). Mei5 and/or Sae3 may substitute for certain Rad51 accessory factors, or these meiotic proteins may function in a unique capacity. Perhaps a nucleoprotein complex consisting of Dmc1, Mei5, and Sae3 is particularly well equipped to promote strand exchange in the context of meiosis-specific chromosome structure. Both Rad51 and Dmc1 appear to act at most DSB sites, raising

the possibility that Mei5 and Sae3 play a role in coordinating the activities of these two recombinases. In this regard, it is interesting to note that the fission yeast Swi5 protein (a Sae3 homolog) has been shown to interact with Rph51 (fission yeast Rad51; Akamatsu *et al.* 2003). If budding yeast Sae3 can interact with both Dmc1 and Rad51, then it may serve as a bridge between these proteins.

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