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Analysis of Close Stable Homolog Juxtaposition During Meiosis in Mutants of *Saccharomyces cerevisiae*

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ABSTRACT

A unique aspect of meiosis is the segregation of homologous chromosomes at the meiosis I division. The pairing of homologous chromosomes is a critical aspect of meiotic prophase I that aids proper disjunction at anaphase I. We have used a site-specific recombination assay in *Saccharomyces cerevisiae* to examine allelic interaction levels during meiosis in a series of mutants defective in recombination, chromatin structure, or intracellular movement. Red1, a component of the chromosome axis, and Mnd1, a chromosome-binding protein that facilitates interhomolog interaction, are critical for achieving high levels of allelic interaction. Homologous recombination factors (Sae2, Rdh54, Rad54, Rad55, Rad51, Sgs1) aid in varying degrees in promoting allelic interactions, while the Srs2 helicase appears to play no appreciable role. Ris1 (a *SWI2/SNF2* related protein) and Dot1 (a histone methyltransferase) appear to play minor roles. Surprisingly, factors involved in microtubule-mediated intracellular movement (Tub3, Dhc1, and Mlp2) appear to play no appreciable role in homolog juxtaposition, unlike their counterparts in fission yeast. Taken together, these results support the notion that meiotic recombination plays a major role in the high levels of homolog interaction observed during budding yeast meiosis.

MEIOSIS is the process by which a parent diploid cell undergoes one round of DNA replication followed by two rounds of chromosome segregation to yield haploid gametes. A unique aspect of meiosis is the segregation of homologous chromosomes at the first meiotic division. Nondisjunction, or improper segregation of homologs, at this stage can lead to gamete aneuploidy, which is a major cause of birth defects in humans (Hassold and Hunt 2001). Homologs are able to correctly orient toward opposite poles of the meiosis I spindle, because a collaboration of DNA crossovers (CR) with sister-chromatid cohesion forms temporary connections between the homologs (Page and Hawley 2003; Petronczki *et al.* 2003).

Homologous chromosomes form progressively stronger associations as cells proceed through meiotic prophase I (Zickler and Kleckner 1999; Storlazzi *et al.* 2003). In the budding yeast, plants, and mammals, *trans*-acting factors required for meiotic recombination are crucial for the pairing and crossing over between homologous chromosomes. In contrast, synaptonemal complex (SC) formation, meiotic nuclear reorganization, and an achiasmate segregation system appear to play supplementary roles in meiotic homolog pairing in the budding yeast (Loidl *et al.* 1994; Weiner and Kleckner 1994; Nag *et al.* 1995; Roeder 1997; Zickler and Kleckner 1998, 1999; Scherthan 2001; Burgess 2002; Peoples *et al.* 2002; Kemp *et al.* 2004).

Several approaches have been taken to analyze nuclear organization and the dynamics of homolog pairing in the budding yeast. The most commonly used method involves fluorescence *in situ* hybridization (FISH) of either intact or spread nuclei. Measurement of the relative proximity of homologous *vs.* nonhomologous sites reports a strong meiosis-induced increase in the proximity of homologous sites up until the first meiotic division (Weiner and Kleckner 1994). Although the deletion of meiosis-specific recombination genes inhibits wild-type levels of meiotic homolog pairing as assayed by FISH, pairing interactions are not completely abolished (Loidl *et al.* 1994; Weiner and Kleckner 1994; Nag *et al.* 1995; Rockmill *et al.* 1995). Thus data obtained using FISH analysis provide a sensitive reporter on allelic interactions between homologous chromosomes in the presence or absence of recombination.

Genetic approaches to dissecting nuclear organization in the budding yeast have also been undertaken. Gene conversion frequencies of heteroalleles inserted at allelic and ectopic sites throughout the budding yeast genome have revealed several aspects of chromosome structure and nuclear organization. First, a

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recombination position effect creates variation in allelic gene conversion frequencies along chromosomes (Lichten and Haber 1989), which correlates with the level of meiotic DNA double-stranded break (DSB) formation around heteroalleles and regional percentage of GC base composition surrounding insertion sites (Borde *et al.* 1999; Petes and Merker 2002). Second, the efficiency of ectopic recombination decays as a function of chromosomal distance between the assayed sites and is still further reduced for sites on different chromosomes (Goldman and Lichten 1996), suggesting that homologs are roughly coaligned at the time of ectopic recombination. Third, impairing normal processes of homolog pairing by introduction of homeologous chromosomes or an *ndj1* mutation increases the rate of ectopic gene conversion (Goldman and Lichten 2000), suggesting that ongoing allelic recombination restricts the chance for ectopic recombination. Furthermore, studies have revealed a role for chromosomal position relative to the telomere in the efficiency of ectopic recombination (Goldman and Lichten 1996; Schlecht *et al.* 2004). Although heteroallelic gene conversion analysis has provided important insights into the architecture of the meiotic nucleus, using gene conversion as both a reporter of relative nuclear position and a measure of recombination rate confounds any inference about the relationship between allelic interactions and homologous recombination.

Exogenous site-specific recombination has also been used to genetically dissect nuclear architecture and meiotic homolog pairing, offering an independent quantitative genetic assay to measure homolog associations in intact living cells of yeast. Cre recombinase is sufficient and necessary to induce *loxP* recombination, the frequency of which depends upon the local concentration of *loxP* sites *in vivo* (Hildebrandt and Cozzarelli 1995). Thus the frequency of Cre-mediated *loxP* recombination (which we term "collisions" to distinguish them from endogenous meiotic recombination events) in a culture reflects the relative proximity and/or accessibility of the *loxP* sites to each other. Induction of meiosis dramatically increases the frequency of collisions between allelic sites, while ectopic collisions remain relatively constant (Peoples *et al.* 2002). The level of allelic collisions observed during wild-type meiosis appears to report on a more stable associated state of homologous chromosomes than that reported by FISH (Peoples *et al.* 2002). In a few mutant situations, homolog pairing levels reported by FISH are relatively unchanged from wild type, whereas allelic interactions reported by the collision assay are strongly reduced (Peoples *et al.* 2002; Peoples-Holst and Burgess 2005). We have termed the high level of allelic interaction during meiosis that is reported by the collision assay "close stable homolog juxtaposition" (CSHJ).

In contrast to using heteroallelic gene conversion as a reporter of homolog pairing, the collision assay does not depend on the endogenous DSB repair machinery *per se*, since ectopic collision levels usually remain unchanged in mutants with severe defects in homologous recombination that have substantial decreases in CSHJ (Peoples *et al.* 2002). Instead, ongoing meiotic recombination may encourage Cre-mediated recombination at allelic *loxP* sites by bringing homologous chromosomes into a progressively more close and stable configuration (J. C. Mell, B. L. Weinholz and S. M. Burgess, unpublished results). Nevertheless, the frequency of allelic collisions is the same in the presence or absence of an ectopic *loxP* site, indicating that ectopic collisions do not compete with allelic collisions (Peoples *et al.* 2002). Given that Cre-mediated *loxP* recombination does not involve an active homology-sensing mechanism, there is little reason to expect that defects in CSHJ would lead to increases in ectopic collisions, as is the case for heteroallelic recombination (Goldman and Lichten 2000). Furthermore, analysis of collisions during vegetative growth in the budding yeast has revealed features of nuclear organization that do not depend on recombination, including the Rabl orientation and high levels of intrachromosomal interactions (Burgess and Kleckner 1999). Thus, the collision assay appears well suited to analyze the effect of mutations on the spatial proximity of allelic sites.

We have previously used the collision assay to show that *trans*-acting factors involved in the initiation and repair of meiotic DSBs make major contributions to wild-type levels of allelic interactions. Deletion of genes involved in the early steps of meiotic recombination, such as initiation of recombination (*spo11*Δ, *rec104*Δ, and *hop1*Δ) and strand invasion (*dmc1*Δ and *hop2*Δ), yield low levels of allelic collisions (Peoples *et al.* 2002). Intermediate CSHJ defects are observed in deletion mutants with defects in directing DSB repair to form crossover products via double Holliday junction intermediates (*zip3*Δ, *mer3*Δ, and *zip2*Δ). Mutants with defects involved in late steps of recombination (*msh4*Δ and *msh5*Δ) do not exhibit any CSHJ defect, suggesting that the creation of joint molecules, not the formation of recombinant products, is of primary importance in achieving wild-type levels of allelic interaction (Peoples-Holst and Burgess 2005). Reduced levels of allelic interactions can reflect altered progression through early and intermediate steps of meiotic recombination, as well as delays or failures to enter into meiosis (as observed in *ime2*Δ).

In this study, we report the CSHJ phenotype of additional mutants using the collision assay to measure allelic and ectopic interactions during the course of meiotic prophase I in the budding yeast. We set out to determine the contributions to CSHJ by factors involved in the following cellular processes: meiotic chromosome structure/recombination initiation, DSB repair, chromatin modification, and microtubule-mediated chromosomal movement.

MATERIALS AND METHODS

Media: YP (1% yeast extract, 2% Bacto–peptone) was supplemented to make the following media types: YPD (2% dextrose, 0.004% tryptophan, and 0.01% adenine sulfate), YPD–ade (2% dextrose, 0.004% tryptophan), YPA (1% potassium acetate, 0.004% tryptophan, and 0.01% adenine sulfate), and YPG (3% glycerol, 0.004% tryptophan, and 0.01% adenine sulfate). Solid media was made by adding 2% Bacto–agar before autoclaving. Sporulation media (SPM; 1% potassium acetate, 0.02% raffinose, 0.1× amino acid mix) and SC–ura media were prepared as described in Burke *et al.* (2000).

Yeast strains: All yeast strains are isogenic derivatives of SK1 (Kane and Roth 1974). Parental haploid strains SBY1338 (*MAT***a** *ho::hisG lys2 ura3Δ::hisG leu2::hisG ade2Δ::hisG trp1::hisG GAL3 flo8::LEU2-loxP-ura3 ndt80Δ::LEU2-loxP-ade2*) and SBY1448 (*MAT*α *ho::hisG lys2::GAL1-Cre-LYS2 ura3Δ::hisG leu2::hisG ade2Δ::hisG trp1::hisG GAL3 flo8::LEU2-pGPD1-loxP-lacZ ndt80Δ::LEU2*) were used for transformation to generate PCR-mediated knockouts (Peoples *et al.* 2002). The *loxP* sites were chosen to be on intermediate-sized chromosomes equidistant between centromere and telomere, and the Cre recombinase was supplied from a galactose-inducible promoter.

For *mnd1Δ::kanMX4*, knockout mutations in SBY1338 and SBY1438 were generated by transformation using PCR-based disruption that replaced the entire open reading frame with the *kanMX4* marker (Wach *et al.* 1994). For *red1Δ::kanMX4*, *mnd1Δ::kanMX4*, *msc1Δ::kanMX4*, *sae2Δ::kanMX4*, *rad55Δ::kanMX4*, *rad51Δ::kanMX4*, *rad54Δ::kanMX4*, *rdh54Δ::kanMX4*, *srs2Δ::kanMX4*, *sgs1Δ::kanMX4*, *ris1Δ::kanMX4*, *dot1Δ::kanMX4*, *tub3Δ::kanMX4*, *dhc1Δ::kanMX4*, and *mlp2Δ::kanMX4* strains, knockouts were made similarly, except that previously constructed knockout strains were purchased from Research Genetics (Huntsville, AL) and PCR primers were designed to amplify regions ~200 bp upstream and downstream of the disrupted open reading frames for use in transformation. All knockouts were confirmed by PCR confirmation of integration of the *KanMX4* marker into the appropriate genomic location and loss of wild-type markers. The *rdh54Δ::kanMX4 rad54Δ::hphMX4* double-mutant strain was generated using PCR-based disruption that replaced the entire open reading frame of *RAD54* with the *hphMX4* marker in the *rdh54Δ::kanMX4* single mutant (Goldstein and McCusker 1999). Diploids used in this study were constructed by crossing the SBY1338- and SBY1438-derived knockout strains.

The parental strain NKY 3230 [also known as SBY 1903; *ho::hisG/ho::hisG leu2::hisG/leu2::hisG ura3/ura3 his4-x::LEU2-(NBam)URA3/HIS4::LEU2-(NBam)*] was used for meiotic division timing and physical analysis of recombination (Hunter and Kleckner 2001). The *dot1Δ::kanMX* and *ris1Δ::kanMX* mutants were generated by PCR-based disruption replacing the entire open reading frame of *RIS1* or *DOT1* with the *kanMX4* marker in NKY 3230. The *ris1Δ::kanMX* mutant was crossed to strains containing *spo11Δ::kanMX* to generate *ris1Δ::kanMX spo11Δ::kanMX*.

Meiotic time courses: Meiotic cell culture synchronization was performed as previously described by Peoples *et al.* (2002) and Padmore *et al.* (1991). For the *rad54Δ rdh54Δ* double mutant, YPD cultures were diluted to $OD_{600} = 2.3$ in YPA media, instead of $OD_{600} = 0.23$.

Collision assay: Kinetic analysis of Cre-mediated *loxP* recombination was carried out by return to mitotic growth (RTG) onto appropriate media and evaluation of the ratio of Ura^+ and Ade^+ prototroph formation to colony-forming units (CFUs) at each time point (reporting on allelic and ectopic *loxP* recombination, respectively) as previously described by Peoples *et al.* (2002). Transfer of cells to SPM marks $t = 0$ of the meiotic time course. Expression of Cre recombinase was induced with the addition of 0.03% galactose at $t = 1$ hr. Sample aliquots were pulled from the culture at $t = 1$ (before induction), 2, 4, 6, 8, and 10 hr. Cell aliquots were pelleted, resuspended in 2% glucose, sonicated 5 sec at 15% maximum power using the microtip of a 550 Sonic ZD-dismembrator (Fisher Scientific), and diluted appropriately prior to plating on selective and nonselective media. RTG viability was determined as CFU at a given time point relative to CFU at $t = 1$ hr. All data shown in this work represent time courses performed with three independent cultures of each strain monitored in parallel with a wild-type control strain. Results have been reproduced in one or more additional independent trials performed in triplicate.

Ectopic collisions were not assessed in the *rad54Δ* single nor in the *rad54Δrdh54Δ* double mutant; colonies formed by these mutant cells exhibited pronounced red/white variegation on YPD–ade plates, even in the absence of galactose-induced expression of Cre recombinase.

Molecular analysis: DNA purification, gel electrophoresis, Southern blotting, and detection of recombination intermediates at the *HIS4::LEU2* recombination hotspot were performed as described in Hunter and Kleckner (2001), except DNA was not psoralen crosslinked. Hybridizing species were quantified using a Storm Phosphorimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Fluorescence *in situ* hybridization: Meiotic nuclear spreads, labeling of probes, and visualization of nuclei were the same as reported in Peoples *et al.* (2002). Alexa-488-labeled cosmid probe *q* (ATCC70891, American Type Culture Collection, Manassas, VA) hybridizes to chromosome *VIII* while Cy3-labeled cosmid probe *g* (pUKG141; B. Dujon, Institut Pasteur, Paris) identifies chromosome *XI*. Hybridization and washing of probes was carried out as described in Weiner and Kleckner (1994).

Spore viability: The *ris1Δ* mutant (*ho::hisG/ho::hisG lys2/lys2 leu2::hisG/leu2::hisG GAL3/GAL3 ris1Δ::kanMX4/ris1Δ::kanMX4*) was patched on YPG for 15 hr, streaked for single colonies on YPD for 2 days, and patched overnight on SPM. After suspension in 20% sorbitol, tetrads were digested with zymolyase, dissected onto YPD plates, and incubated at 30° for 2 days.

Meiosis I division timing: The timing and synchrony of the first meiotic division was determined by staining cells with DAPI and evaluating whether cells contained one or more than one nuclear focus by light microscopy. At least 200 cells were counted for each time point.

RESULTS AND DISCUSSION

We used a previously described quantitative genetic assay based on Cre/*loxP* site-specific recombination to measure the relative proximity and/or accessibility of allelic and ectopic sites during the course of meiotic prophase I in the budding yeast (Peoples *et al.* 2002). Allelic and ectopic loci in diploid yeast were modified so that Cre-mediated recombination (collisions) between allelic or ectopically positioned *loxP* sites results in the formation of either Ura^+ (*pGPD1-loxP-ura3*) or Ade^+ (*pGPD1-loxP-ade2*) prototrophs, respectively, upon RTG from sporulating cultures. Prototrophs arise when collisions place the constitutive promoter (*pGPD1*) linked to a *loxP* site on the same chromatid linked to either a *loxP:ura3* construct residing on the homolog or a *loxP:ade2* construct located at an ectopic position on a nonhomologous chromosome.

The maximum level of allelic collisions (between $t = 8$ and 10 hr) is 13-fold higher than the ectopic collision frequency during wild-type meiosis and defines CSHJ. CSHJ defects conferred by mutations affecting a variety of aspects of meiosis I prophase are easily discriminated (Peoples *et al.* 2002; Peoples-Holst and Burgess 2005; this work). The collision assay was carried out in an *ndt80*Δ background (referred to here as "wild type" for collision assay experiments). The *ndt80*Δ mutant arrests in pachytene with full-length SC and unresolved double Holliday junction intermediates (Xu *et al.* 1995; Allers and Lichten 2001). It should be noted that, by performing experiments in the *ndt80*Δ background, the length of meiotic prophase may be extended compared to *NDT80* and thus select for more stable associations (Peoples *et al.* 2002). The use of the *ndt80*Δ mutation allowed for recovery of cells in most mutants via RTG that would otherwise be inviable if allowed to complete meiotic divisions and sporulate (Sherman and Roman 1963; Esposito and Esposito 1974; Zenvirth *et al.* 1997). The RTG viability of the *ndt80*Δ single mutant and previously analyzed mutants by the collision assay do not decrease over the course of 10 hr in SPM; here we report on a set of mutants that exhibit defects in RTG viability. In this study, we evaluated the CSHJ phenotype of a series of deletion mutants produced in this "wild-type" strain background.

Meiotic chromosome structure and recombination initiation: Meiotic chromosomes are arranged in linear arrays of chromatin loops. The bases of loops form a structural axis that is elaborated during meiotic prophase (Moens and Pearlman 1988; Blat *et al.* 2002). The axes of two homologs are ultimately connected along their lengths by the central element of the SC. The progression of structural/axial events occurs in parallel to the DNA events of recombination. Axial elements, which will become the lateral elements of the SC, are formed concomitant with meiotic DSB formation (Padmore *et al.* 1991).

Several proteins localize along the length of the meiotic axial element, including Red1, Hop1, and Mek1 (Smith and Roeder 1997; Bailis and Roeder 1998; de los Santos and Hollingsworth 1999). All are thought to play roles in channeling DSB repair to homologous chromosome repair templates instead of sister chromatids (Hollingsworth *et al.* 1995; Schwacha and Kleckner 1997; Thompson and Stahl 1999). These proteins function at an early stage of meiosis, affecting DSB formation and turnover, homolog pairing, and synapsis (Smith and Roeder 1997; Bailis and Roeder 1998; de los Santos and Hollingsworth 1999; Blat *et al.* 2002). Other factors that form discrete foci along the meiotic axis (*e.g.*, *HOP2*, *MND1*) are also presumed to play important roles in facilitating recombination between homologous chromosomes over sister chromatids (Gerton and DeRisi 2002; Tsubouchi and Roeder 2002; Zierhut *et al.* 2004). We have previously shown that allelic Cre/*loxP* collisions are strongly reduced in *hop1*Δ and *mek1*Δ mutants relative to wild type (Peoples *et al.* 2002). While *hop1*Δ and *red1*Δ mutants show *spo11*Δ-like defects in CSHJ, *mek1*Δ has a slightly less severe phenotype (Peoples *et al.* 2002). Here we report the effects of additional mutations in genes that have putative roles in homolog partner choice: *red1*Δ, *mnd1*Δ, and *msc1*Δ.

*red1*Δ*:* The *red1*Δ mutant exhibits severe defects in SC formation (Rockmill and Roeder 1990), has substantially reduced homolog pairing (~31% of wild type) when assayed using FISH (Nag *et al.* 1995), makes reduced levels of meiotic DSBs (Xu *et al.* 1997), and reduces the number of recombination events between homologs (Schwacha and Kleckner 1997). Deletion of *RED1* alleviates the arrest of a *dmc1*Δ mutant; presumably by allowing DSB repair from sister chromatids (Schwacha and Kleckner 1997; Xu *et al.* 1997; Bishop *et al.* 1999).

In the *red1*Δ mutant, we found that allelic Cre/*loxP* collisions were strongly reduced to approximately the same level observed for *spo11*Δ, while no change in the level of ectopic collisions was observed (Figure 1A). The *red1*Δ mutant exhibited wild-type RTG viability by 10 hr in sporulation medium (Figure 1B).

The formation of axial elements is severely impaired in the *red1*Δ mutant, whereas *hop1*Δ and *mek1*Δ mutants have partial development of axial elements (*red1*Δ > *hop1*Δ > *mek1*Δ) (Rockmill and Roeder 1990). Recent evidence suggests that Red1 acts as a structural scaffold for Hop1, which activates the Mek1 kinase in response to local meiotic DSB formation, presumably recruiting recombination factors to the DSB site (Wan *et al.* 2004; Niu *et al.* 2005) and mediating recombination partner choice via the axis (Blat *et al.* 2002). One explanation for the less severe *mek1*Δ CSHJ defect compared with *red1*Δ and *hop1*Δ is that there is another effector in addition to Mek1 downstream of Red1 and Hop1 that stimulates partner choice, allowing for a small increase in allelic collisions above DSB-independent levels in *mek1*Δ. Alternatively, the difference between *mek1*Δ and *red1*Δ/*hop1*Δ mutants may partially reflect different defects in maximal levels of DSB formation (*hop1*Δ at ~5–10% of wild type, *red1*Δ at ~50%, and *mek1*Δ at ~100%) (Xu *et al.* 1997; Woltering *et al.* 2000; Blat *et al.* 2002; Pecina *et al.* 2002).

*mnd1*Δ*: MND1* is required for normal meiotic recombination and meiotic prophase I progression along with its partner Hop2 (Gerton and DeRisi 2002; Tsubouchi and Roeder 2002). Mnd1 and Hop2 help drive recombination between allelic instead of ectopic positions (Tsubouchi and Roeder 2002; Zierhut *et al.* 2004). Although Mnd1 does not colocalize with Rad51 foci and its association with chromosomes does not depend on DSB formation, Mnd1 and Hop2 may facilitate homolog partner choice through their roles in meiotic chromosome structure (Zierhut *et al.* 2004) or indirectly by stimulating strand exchange (Petukhova

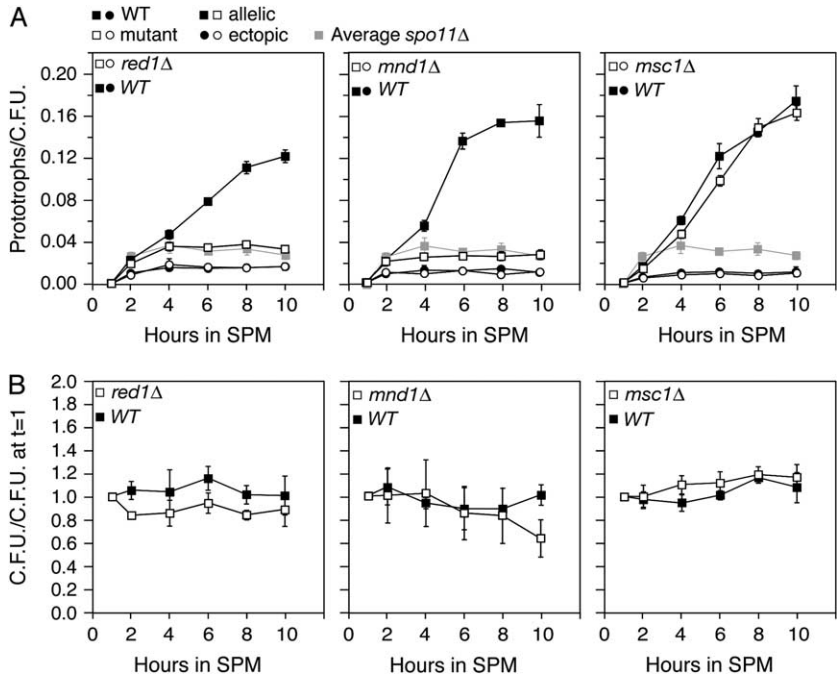

Figure 1.—Commitment to Cre-mediated *loxP* recombination in wild-type (*ndt80*Δ in this study) and isogenic strains deleted for *RED1* (left), *MND1* (middle), or *MSC1* (right). (A) Cells synchronized to enter meiosis were plated on selective and nonselective media following Cre induction at $t = 1$ hr after meiotic induction. Allelic collisions (squares) are reported by Ura$^+$ prototrophs/CFU and ectopic collisions (circles) are reported by Ade$^+$ prototrophs/CFU. The wild-type control is indicated by solid squares and circles; mutants are designated by open squares and circles. Measurements shown are the average of three independent cultures; error bars indicate the standard deviation from the mean. Shaded squares indicate averaged *spo11*Δ allelic interaction values from nine independent cultures (Peoples-Holst and Burgess 2005). (B) Return-to-growth viability monitors survival throughout the meiotic time courses shown in A. Viability is represented as CFU on nonselective media at each time point divided by the CFU for that strain at the $t = 1$ hr time point prior to Cre induction. The wild-type control is indicated by solid squares; mutants are designated by open squares.

et al. 2005). Using GFP-tagged chromosomes, genetic epistasis analysis has demonstrated that *MND1* acts in the same pathway as *DMC1* to facilitate homolog pairing (Chen *et al.* 2004).

We have found that both the *mnd1*Δ and the *hop2*Δ mutants exhibited CSHJ defects similar to that of *spo11*Δ, while ectopic collision levels remained unchanged relative to the wild-type control (Figure 1A; Peoples *et al.* 2002). Although Hop2 and Mnd1act together as a heterodimer to promote strand exchange and each requires the other to localize to chromosomes (Tsubouchi and Roeder 2002), *mnd1*Δ shows a late decrease in RTG viability (Figure 1B), whereas *hop2*Δ exhibits wild-type levels of RTG viability (Peoples *et al.* 2002). *In vitro* work has shown that mouse Hop2 by itself has strand-exchange activity, while mouse Mnd1 inhibits the D-loop formation mediated by Hop2 (Petukhova *et al.* 2005). Perhaps in the absence of Mnd1, Hop2 causes the formation of poisonous recombination intermediates, leading to a slight decrease in RTG viability.

*msc1*Δ*: MSC1*, along with *RED1*, was identified in a screen for factors that direct meiotic DSBs to homologous chromosomes rather than sister chromatid repair substrates (Thompson and Stahl 1999). Due to phenotypic similarity to the *dmc1*Δ with respect to high levels of sister-chromatid exchange, the *msc1* mutant was originally thought to be part of the *DMC1*-promoted homolog exchange pathway (Thompson and Stahl 1999). More recently, however, it has been reported that a different disruption of *MSC1* did not recapitulate the sister-chromatid recombination phenotype, although this allele did exhibit mitotic chromosome instability (Thompson and Stahl 2003). When we examined the *msc1*Δ deletion mutant, allelic and ectopic collision levels, as well as RTG viability, were indistinguishable from that of wild type (Figure 1). Therefore, we found that Msc1 plays no apparent role in CSHJ.

Early DSB processing: Meiotic recombination in the budding yeast occurs through the programmed formation and repair of DNA DSBs, involving both general DSB repair enzymes as well as meiosis-specific factors (Keeney 2001). Our previous studies using the Cre/*loxP* collision assay focused primarily on meiosis-specific genes involved in recombination. Here we evaluated the effect of mutations that affect the processing of meiotic DSBs into downstream recombination intermediates and also play a role in DNA repair in vegetative cells. We report the allelic and ectopic Cre/*loxP* collisions and RTG viability during meiotic prophase I in the following mutants: *sae2*Δ, *rad55*Δ, *rad51*Δ, *rad54*Δ, *rdh54*Δ, and *rad54*Δ *rdh54*Δ.

*sae2*Δ*:* The product of *SAE2*, also known as *COM1*, acts early in meiotic DSB repair; the *sae2*Δ mutant forms Spo11-induced meiotic DSBs, but Spo11 remains attached to the 5′-termini of DSBs and subsequent repair steps are blocked (McKee and Kleckner 1997; Prinz *et al.* 1997). The *sae2*Δ mutant exhibits 25% of wild-type homolog pairing levels by FISH (Prinz *et al.* 1997). Since *sae2*Δ mutants exhibit slight sensitivity to MMS and increased HO endonuclease-induced mutation rate but no defect in mitotic heteroallelic recombination, Sae2 appears to play a minor role in mitotic DSB repair (McKee and Kleckner 1997; Rattray *et al.* 2001).

We found that the *sae2*Δ mutant exhibited strongly reduced allelic collisions during meiosis, although still

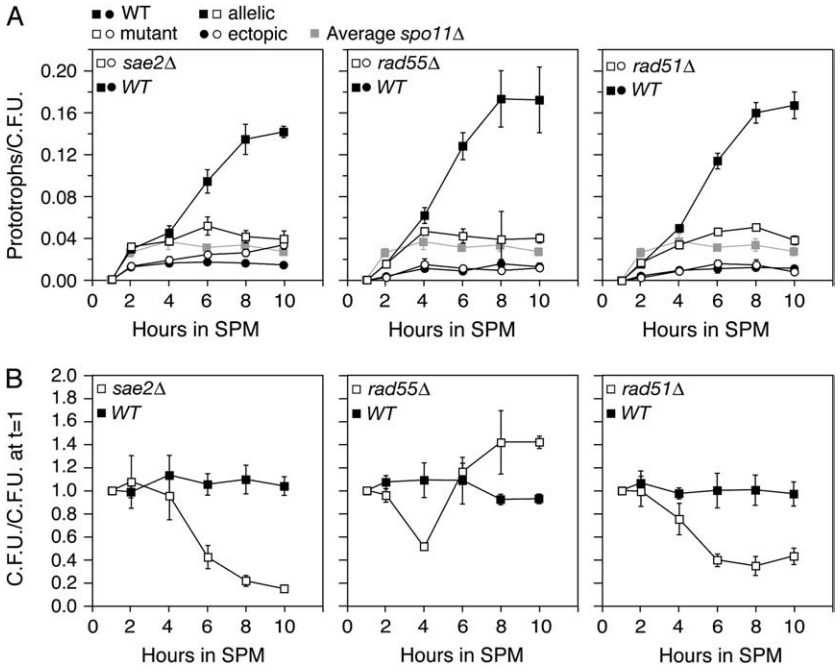

FIGURE 2.—Commitment to Cre-mediated *loxP* recombination in wild-type and isogenic strains deleted for genes involved in early DSB processing: *SAE2* (left), *RAD55* (middle), or *RAD51* (right). Graph parameters are the same as in Figure 1: (A) Allelic and ectopic collision levels. (B) Return-to-growth viability for time courses in A.

elevated above *spo11*Δ allelic collision levels (Figure 2A). Interestingly, the ectopic collision level observed for the *sae2*Δ was increased above that of the wild-type strain at later time points (Figure 2A). This is the first mutant, aside from *ime2*Δ, which does not enter meiosis, in which we have observed this phenotype.

The viability of the *sae2*Δ mutant during RTG was decreased to ~15–30% of the wild-type level by the 10-hr time point (Figure 2B; McKee and Kleckner 1997; Prinz *et al.* 1997). Perhaps mechanisms to remove Spo11 from DSB ends are unavailable during RTG, causing failure in DSB repair and thus decreased viability.

The decrease in RTG viability in the *sae2*Δ mutant could select for a subset of surviving cells at later time points in sporulation. This could confound a direct interpretation of the Cre/*loxP* collision phenotype with respect to the mildly increased allelic collisions relative to *spo11*Δ and the mildly increased ectopic collisions relative to wild type. However, 5′–3′ resection of meiotic DSB ends to expose single-stranded (ssDNA) tails is critical to subsequent steps of homologous recombination. Thus our results with this mutant are consistent with a role for processing of meiotic DSB ends into downstream recombination intermediates in achieving CSHJ. Preliminary quantitative PCR data measuring Cre/*loxP* recombinants in cells progressing through meiosis suggest that the allelic collision levels of the total population are reduced similarly to the surviving subpopulation after RTG for the *sae2*Δ, *rad55*Δ, and *rad51*Δ mutants (D. Y. Lui and S. M. Burgess, unpublished data).

*rad55*Δ*: RAD55* was identified as a factor involved in the repair of DNA damage induced by ionizing radiation and plays a role in meiotic recombination (Lovett and Mortimer 1987). Rad55 and its partner Rad57 promote the assembly of Rad51 nucleoprotein filaments by actively loading it onto ssDNA tracts bound by replication protein A (RPA) (Gasior *et al.* 2001).

We examined a *rad55*Δ mutant during meiosis to ascertain the effect of reduced loading of Rad51 onto ssDNA on homolog juxtaposition. Allelic collision levels were decreased in the *rad55*Δ mutant to nearly the level exhibited by *spo11*Δ. Ectopic levels were unchanged relative to the wild-type control (Figure 2A). We found *RAD55* to be important for achieving CSHJ, presumably by aiding in the assembly of Rad51 and/or Dmc1 to 3′-ssDNA tails.

Interestingly, the *rad55*Δ mutant had a transient decrease in RTG viability (~50%) at $t = 4$ hr, but then RTG viability returned to ~100% by $t = 6$ hr (Figure 2B). By $t = 8$ hr, there were more colony-forming units than at $t = 1$ hr. This behavior was reproducible in independent experiments. We reason that, in the absence of *RAD55*, RPA-coated ssDNA tails are unable to be repaired during RTG at early points in prophase I, but alternative mechanisms that process these tails become available later in prophase I. The increase in colony-forming units at $t = 8$ hr above that at $t = 1$ suggests that during the first hour of incubation in sporulation medium, ~30% of cells have already lost the ability to survive RTG. This has been shown to be the case in other experiments (D. Y. Lui and S. M. Burgess, unpublished results). In any case, the means by which *rad55*Δ cells recover RTG viability at late time points does not involve a mechanism that rescues the defect in allelic collision levels, suggesting that DSBs are repaired from sister-chromatid repair substrates during RTG.

*rad51*Δ*:* Rad51 and its meiosis-specific paralog Dmc1 are homologs of the bacterial strand-exchange protein

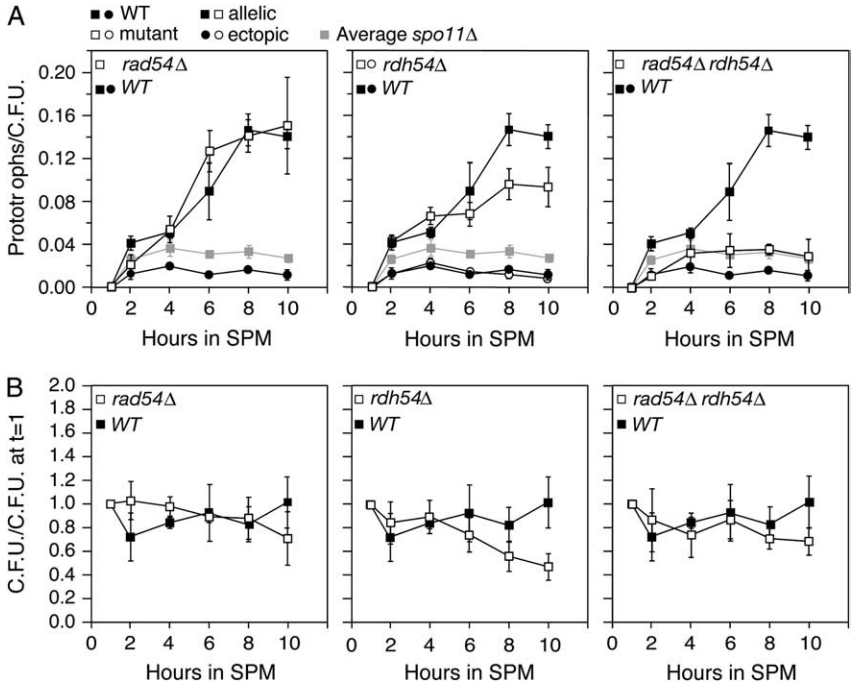

FIGURE 3.—Commitment to Cre-mediated *loxP* recombination in wild-type and isogenic strains deleted for *RAD54* (left), *RDH54* (middle), or both *RAD54* and *RDH54* (right). Graph parameters are the same as in Figure 1: (A) Allelic and collision levels in *rad54*Δ, *rdh54*Δ, and *rad54*Δ *rdh54*Δ and ectopic collisions in *rdh54*Δ. (B) Return-to-growth viability for time courses in A.

RecA (SHINOHARA *et al.* 1992). Rad51 acts in both mitosis and meiosis and forms a helical filament on ssDNA left after resection of a DSB. This nucleoprotein filament then searches the genome for homology to initiate the repair of the DSB by promoting strand exchange and the formation of a D-loop (KROGH and SYMINGTON 2004). Rad51 and Dmc1 contribute independently to meiotic recombination, yet they have some overlapping functions (DRESSER *et al.* 1997; SHINOHARA *et al.* 1997a). Each single mutant and the *dmc1*Δ *rad51*Δ double mutant exhibit reduced levels of homolog pairing (ROCKMILL *et al.* 1995; CHEN *et al.* 2004).

When we examined the *rad51*Δ mutant, allelic collision levels were substantially reduced relative to wild type and slightly elevated above *spo11*Δ (Figure 2A). This is consistent with the *rad51*Δ mutant exhibiting low, but elevated above the *dmc1*Δ mutant, levels of homolog pairing, as monitored in spread chromosome preparations (CHEN *et al.* 2004). Ectopic interactions in the *rad51*Δ mutant were unchanged relative to the wild-type strain (Figure 2A). Unlike in *dmc1*Δ mutants (PEOPLES *et al.* 2002), *rad51*Δ exhibited reduced RTG survivability (~40% of wild-type level; Figure 2B), which likely reflects the role of Rad51, but not Dmc1, in DSB repair during mitotic RTG. The detection of low allelic collision levels in the *rad51*Δ mutant implicates Rad51 in CSHJ, likely by promoting strand invasion of recombinogenic 3′-ssDNA ends. The absence of either protein leads to a *spo11*Δ-like defective CSHJ by the collision assay.

*rad54*Δ *and rdh54*Δ*:* The paralogs *RAD54* and *RDH54* encode proteins in the *SWI2/SNF2* family of chromatin-remodeling factors and play important roles in mitotic and meiotic recombination, respectively (KLEIN 1997; SHINOHARA *et al.* 1997b). *In vitro,* Rad54 facilitates D-loop formation by Rad51-coated ssDNA filaments and can alter the topology of DNA, presumably allowing for the remodeling of chromatin (ALEXEEV *et al.* 2003). During meiosis, Rdh54 is implicated in the colocalization of Rad51 and Dmc1, D-loop formation, and establishment of crossover interference (PETUKHOVA *et al.* 2000; SHINOHARA *et al.* 2000, 2003). Deletion of *RDH54* results in a severe defect in meiotic recombination, while *RAD54* appears to be dispensable (SHINOHARA *et al.* 1997b; SCHMUCKLI-MAURER and HEYER 2000). In contrast, during vegetative growth, the absence of *RAD54* confers severe defects in DSB repair, whereas *RDH54* plays a subtler role (KLEIN 1997). However, the *rad54*Δ *rdh54*Δ double mutant has a more severe meiotic recombination defect than either single mutant, suggesting that the two genes are partially redundant during meiotic prophase I (SHINOHARA *et al.* 1997b).

We found that the *rad54*Δ mutant exhibited wild-type levels of allelic collisions during meiotic prophase I (Figure 3A). RTG viability in the *rad54*Δ mutant was ~70% of wild-type level by the 10-hr time point (Figure 3B). Allelic collisions in the *rdh54*Δ mutant were reduced to an intermediate level between wild type and *spo11*Δ (Figure 3A). RTG viability was ~45% of wild type by $t = 10$ hr (Figure 3B). The *rad54*Δ *rdh54*Δ double mutant exhibited a strong defect in CSHJ, around *spo11*Δ levels of allelic collisions, indicating a synthetic defect when these mutations are combined (Figure 3A). RTG viability of the *rad54*Δ *rdh54*Δ double mutant was less severe than in the *rdh54*Δ single mutant at ~70% of wild type at $t = 10$ hr (Figure 3B).

While we observed some RTG inviability in *rad54*Δ, *rdh54*Δ, and *rad54*Δ *rdh54*Δ mutants, our results contrast sharply with the strong RTG viability defects observed by Shinohara *et al.* (1997b). The lack of a pronounced survival defect in our strains could be due to our use of the *ndt80*Δ mutation. In *ndt80*Δ cells, *rdh54*Δ *rad54*Δ mutants may arrest prior to the formation of recombination intermediates that cannot be adequately repaired under RTG conditions or prior to the formation of poisonous recombination products. As a caveat, *rad54*Δ *rdh54*Δ mutants are known to have strong defects in entry into meiosis; thus this double-mutant CSHJ defect may simply reflect premeiotic levels of allelic collision.

Rdh54 appears to specifically promote crossovers that are subject to interference while Rad54 appears to function in a general repair mechanism (Bishop *et al.* 1999; Shinohara *et al.* 2003). Although *RAD54* and *RDH54* may be partially redundant, the reduced allelic collisions observed in *rdh54*Δ suggest that meiotic recombination events involving Rdh54 play a unique role in CSHJ.

Helicases involved in recombination: We have previously analyzed a mutant in *MER3*, which encodes a 3′–5′ helicase. The *mer3*Δ mutant exhibited intermediate levels of allelic collisions relative to *spo11*Δ and wild type. To determine the contribution to CSHJ by other helicases that have been implicated in recombination, we analyzed *srs2*Δ and *sgs1*Δ mutants.

*srs2*Δ*: SRS2*, also known as *HPR5*, encodes a protein with DNA-dependent ATPase and 3′–5′ helicase activities (Rong and Klein 1993). The *srs2*Δ mutant exhibits a mitotic hyperrecombination phenotype and defects in meiotic spore viability (Palladino and Klein 1992). Biochemical evidence suggests that Srs2 regulates DSB repair by disrupting Rad51 filaments formed on ssDNA (Krejci *et al.* 2003; Veaute *et al.* 2003). An analysis of meiotic recombination in an *srs2*Δ mutant strain has not been reported to our knowledge.

We observed allelic and ectopic collisions in *srs2*Δ that were comparable to those seen in wild type. (Figure 4A). RTG viability was reduced in *srs2*Δ, reaching ~35% viability by $t = 10$ hr, consistent with a repair defect in vegetatively dividing cells upon RTG (Figure 4B). We interpret the lack of a role for Srs2 in CSHJ as an indication that Srs2 does not play a role in the formation or stabilization of joint molecules in meiosis, but the RTG defect suggests that *SRS2* is required for the generation of normal recombination products.

*sgs1*Δ*:* Sgs1, the only RecQ family helicase found in budding yeast, has been implicated in negative regulation of homolog interaction in meiosis, since increased coalignment between homolog axes are observed cytologically in the absence of SC and crossover levels measured genetically are modestly increased in the *sgs1*Δ mutant (Rockmill *et al.* 2003).

We found that allelic interactions in the *sgs1*Δ were reduced relative to wild type but remained higher than

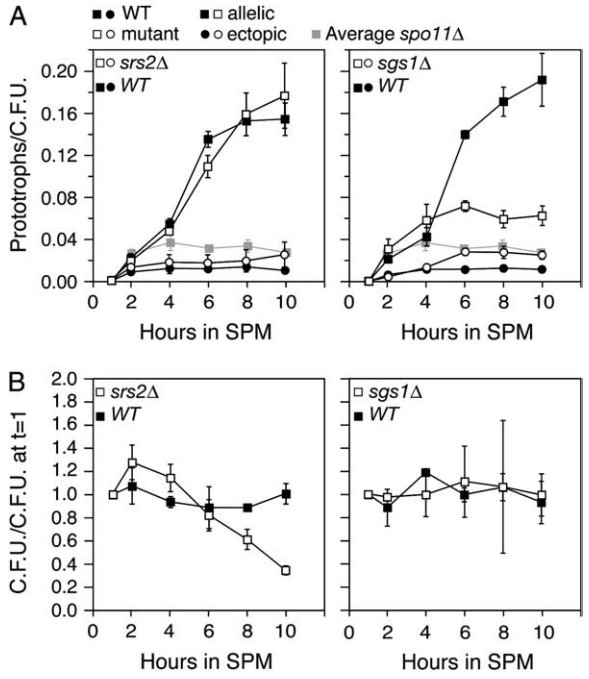

Figure 4.—Commitment to Cre-mediated *loxP* recombination in wild-type and isogenic strains deleted for helicases *SRS2* (left) and *SGS1* (right). Graph parameters are the same as in Figure 1: (A) Allelic and ectopic collisions. (B) Return-to-growth viability for time courses in A.

*spo11*Δ levels (Figure 4A). Interestingly, ectopic collisions were increased (Figure 4A). No change in viability in the *sgs1*Δ was detected throughout the time course (Figure 4B). At $t = 6$ hr into meiosis, FISH analysis was also carried out on the *sgs1*Δ mutant and a wild-type strain and gave lower levels of pairing (the fraction of spread nuclei in which homologous pairs of loci were ≤0.7 μm apart) than exhibited in wild-type strains (Table 1). The *ndt80*Δ ("wild-type") strain gave allelic pairing levels of 0. 74 ± 0.09 and 0.80 ± 0.07 for chromosomes *VIII* and *XI*, respectively, while *sgs1*Δ gave levels of 0.55 ± 0.04 and 0.58 ± 0.11 (Table 1). The background pairing (fraction of spread nuclei exhibiting nonhomologous pairs of loci that were ≤0.7 μm apart) was increased from 0.03 to 0.07 (Table 1), consistent with increased ectopic Cre/*loxP* collisions in the *sgs1*Δ mutant.

Our results suggest that *sgs1*Δ impacts CSHJ only partially. This is reminiscent of mutants such as *zip1*Δ, *zip2*Δ, and *zip3*Δ, which affect the CR-specific branch of the meiotic double-strand break repair pathway (Borner *et al.* 2004; Peoples-Holst and Burgess 2005). Perhaps *SGS1* contributes to only one of the branches. Considering that allelic collision levels are reduced in the *sgs1*Δ mutant, it is curious that there are increased axial associations between homologs in an *sgs1*Δ *zip1*Δ mutant relative to *zip1*Δ (Rockmill *et al.* 2003). Axial interactions observed cytologically may be structurally different from those that promote Cre/*loxP* recombination, possibly

TABLE 1

Pairing levels by FISH for *sgs1*Δ *ndt80*Δ

Genotype	Experiment	Pairing level[a]		n	% wild type
		$VIII_{obs}$; XI_{obs}; bkgd	$VIII_{obs-bkgd}$; $XI_{obs-bkgd}$		
*ndt80*Δ	1	0.70; 0.78; 0.03	0.67; 0.75	93	
*ndt80*Δ	2	0.83; 0.88; 0.03	0.80; 0.85	105	
Average (*VIII*; *XI*)			0.74 ± 0.09; 0.80 ± 0.07		100
*sgs1*Δ *ndt80*Δ	1	0.65; 0.57; 0.07	0.58; 0.50	77	
*sgs1*Δ *ndt80*Δ	2	0.59; 0.72; 0.07	0.52; 0.65	92	
Average (*VIII*; *XI*)			0.55 ± 0.04; 0.58 ± 0.11		73

[a] Pairing assessed at $t = 6$ hr in SPM with fluorescently labeled cosmid probes hybridized to spread nuclei preparations. Observed pairing levels are the fraction of nuclei scored with a distance between foci of ≤0.7 μm. Background pairing levels for each hybridization were determined as the fraction of nuclei scored with nonhomologous pairs of loci that were ≤0.7 μm apart.

representing less stable interactions. Alternatively, contacts formed in the context of the SC may limit the accessibility of *loxP* sites despite close proximity.

Chromatin structure: Chromatin structure has been implicated in chromosome association and progression of meiosis (Peters *et al.* 2001; Sharma *et al.* 2003; Prieto *et al.* 2005; Webster *et al.* 2005). In mice, the lack of the DNA methyltransferase Dmnt3L or the histone methyltransferase Suv39h cause failure of homologous chromosome alignment and synapsis during spermatogenesis (Peters *et al.* 2001; Webster *et al.* 2005). Mice lacking another DNA methyltransferase, Dmnt3a, exhibited a delay in meiotic entry but spermatocytes achieved full levels of synapsis (Yaman and Grandjean 2006). Chromatin structure also plays a role in *Schizosaccharomyces pombe* meiosis, as mutants defective for establishing silenced chromatin exhibit aberrant meioses with defective horsetail movement, reduced recombination, chromosome missegregation, and low spore viability (Nimmo *et al.* 1998; Hall *et al.* 2003). In organisms with achiasmate segregation, chromatin organization into heterochromatic and euchromatic regions during meiosis dictates how chromosomes pair and synapse (Dernburg *et al.* 1996; Karpen *et al.* 1996). Here we evaluated two mutants that exhibit apparently opposite defects. While the *SWI2/SNF2*-like ATPase Ris1 is an antisilencer, the histone methyltransferase Dot1 is implicated as a silencing factor.

*ris1*Δ*: RIS1*, also known as *DIS1* and *TID4*, plays a role in antagonizing silencing, facilitates mating-type switching, and contains an N-terminal domain that interacts with the Sir4-silencing factor (Zhang and Buchman 1997). Inclusion of Ris1 in this analysis was based on its interaction with Dmc1 by two-hybrid (Dresser *et al.* 1997).

We found that allelic collision levels in *ris1*Δ were slightly reduced relative to wild type, but were nonetheless well above *spo11*Δ levels (Figure 5A). Ectopic collisions and RTG viability in *ris1*Δ were unchanged relative to the wild-type strain (Figure 5A). Since the *ris1*Δ mutant had mildly reduced levels of allelic collisions in meiosis, we evaluated additional meiotic phenotypes. We first examined the role of *RIS1* in the kinetics of the meiosis I division and found that *ris1*Δ was delayed relative to wild type (*NDT80)* (Figure 5B). This delay was not suppressed by adding a *spo11*Δ mutation, indicating that the delay caused by *ris1*Δ is not likely due to a failure to process meiotic DSBs. Southern blot analysis of DSB turnover and crossover formation at the *HIS4::LEU2* recombination hotspot revealed that both were delayed (Figure 5B). The *ris1*Δ mutant gave 98% spore viability among 145 dissected tetrads, which is typical of a wild-type (*NDT80*) SK1 strain background, indicating that the role of *RIS1* in high levels of CSHJ and the timing of recombinant formation are not essential for normal progression through the meiotic program. The *ris1*Δ mutation may have delayed entry into meiosis or a defect in progression through an early part of meiosis prior to the formation of DSBs.

*dot1*Δ*: DOT1* encodes a histone methyltransferase that affects telomeric silencing and is implicated in meiotic pachytene checkpoint control (San-Segundo and Roeder 2000; Lacoste *et al.* 2002; Ng *et al.* 2002; van Leeuwen *et al.* 2002). This mutation has been shown to give nearly wild-type levels of spore viability and crossing over (San-Segundo and Roeder 2000).

In the *dot1*Δ mutant, we found that CSHJ was attained albeit with a 2-hr delay compared to wild type (Figure 6A). Ectopic collisions and RTG viability in the *dot1*Δ mutant were similar to wild type (Figure 6A). We analyzed the timing of the meiosis I division and recombination intermediates in the *dot1*Δ mutant to further refine its meiotic phenotype (Figure 6B). Crossover formation at the *HIS4::LEU2* hotspot and the meiosis I division—but not DSB formation—were delayed, implicating a role for *DOT1* in late meiotic prophase. The delay in achieving CSHJ and timely

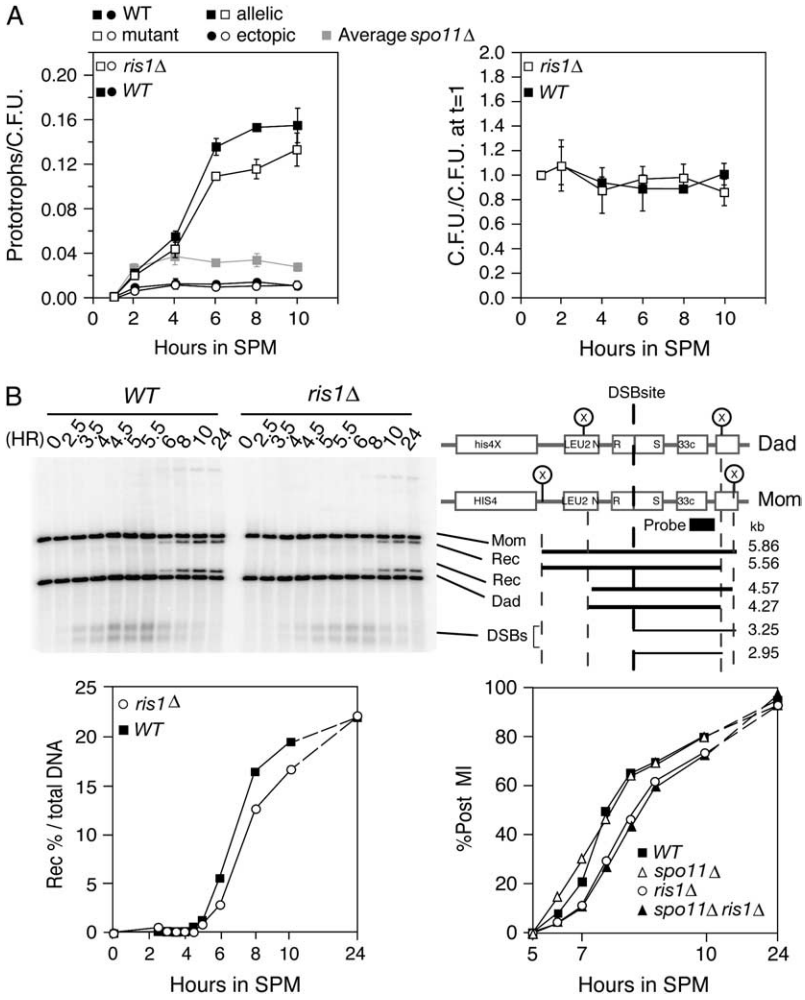

FIGURE 5.—Commitment to Cre-mediated *loxP* recombination and physical analysis of meiotic recombination at the *HIS4::LEU2* hotspot in wild-type and isogenic strains deleted for *RIS1*. (A) Allelic and ectopic collisions in wild-type (*ndt80*Δ) and the isogenic *ris1*Δ strains (left) with the return-to-growth viability for the time course (right). Graph parameters are the same as in Figure 1. (B) In a separate time course, recombinant formation at the *HIS4::LEU2* hotspot was analyzed by Southern blot (top) and bands corresponding to both recombinants in wild-type (*NDT80*) (solid boxes) and *ris1*Δ (open circles) were quantified (bottom left). The timing of the meiosis I division (bottom right) was also determined in wild-type (*NDT80*) (solid squares), *ris1*Δ (open circles), *spo11*Δ (open triangles), and *ris1*Δ *spo11*Δ (solid triangles).

crossover formation suggests that the *dot1*Δ mutation may alter the transition from DSB to stable recombination intermediates, which promote allelic collisions.

Movement-associated factors Dhc1, Tub3, and Mlp1 do not contribute to CSHJ: Meiotic prophase is notable for the dynamic chromosomal movements in many species (LOIDL 1990; DERNBURG *et al.* 1995; HIRAOKA 1998; DAVIS and SMITH 2001). In particular, the clustering of telomeres at the nuclear envelope at the "bouquet" stage has been postulated to reduce the homology search from three dimensions to two dimensions (ROEDER 1997; ZICKLER and KLECKNER 1998). Although *NDJ1* is required for formation of the bouquet and the timely achievement of CSHJ in budding yeast, the role of *NDJ1* in CSHJ depends on meiotic recombination, facilitating the transition from single-end invasions to double Holliday junctions (PEOPLES-HOLST and BURGESS 2005; WU and BURGESS 2006). In the fission yeast *S. pombe*, dynein-dependent oscillatory movements of the nucleus led by the telomeres and the spindle-pole body are important for the efficiency of meiotic homolog pairing and recombination (CHIKASHIGE *et al.* 1994; COOPER *et al.* 1998; YAMAMOTO *et al.* 1999; MIKI *et al.* 2002; DING *et al.* 2004). In plants, colchicine, a drug that inhibits the polymerization of microtubules, affects synapsis in maize (COWAN and CANDE 2002). To further assess how active nuclear movements may affect homolog pairing in the budding yeast, we analyzed mutants defective for α-tubulin, dynein heavy chain, and a myosin-like protein.

*tub3*Δ*: TUB3* encodes one of the two α-tubulin genes from budding yeast (SCHATZ *et al.* 1986a). We chose to examine a *tub3*Δ strain as this mutation has been shown to be nonessential for meiosis yet exhibit poor spore viability (SCHATZ *et al.* 1986b). In the *tub3*Δ mutant, we observed that allelic and ectopic collisions were indistinguishable from the wild-type control (Figure 7A) and that RTG viability remained constant in the *tub3*Δ mutant (Figure 7B).

It is possible that the other α-tubulin encoded by *TUB1* substitutes for *TUB3* function. Cellular defects caused by a null mutation in either of these genes can be suppressed by extra copies of the nonidentical gene (SCHATZ *et al.* 1986b). Nonetheless, the two α-tubulins have been shown to play different roles microtubule dynamics (BODE *et al.* 2003). Taken together, these data indicate that *TUB3* does not play a role in CSHJ. Consistent with these data, results from our laboratory

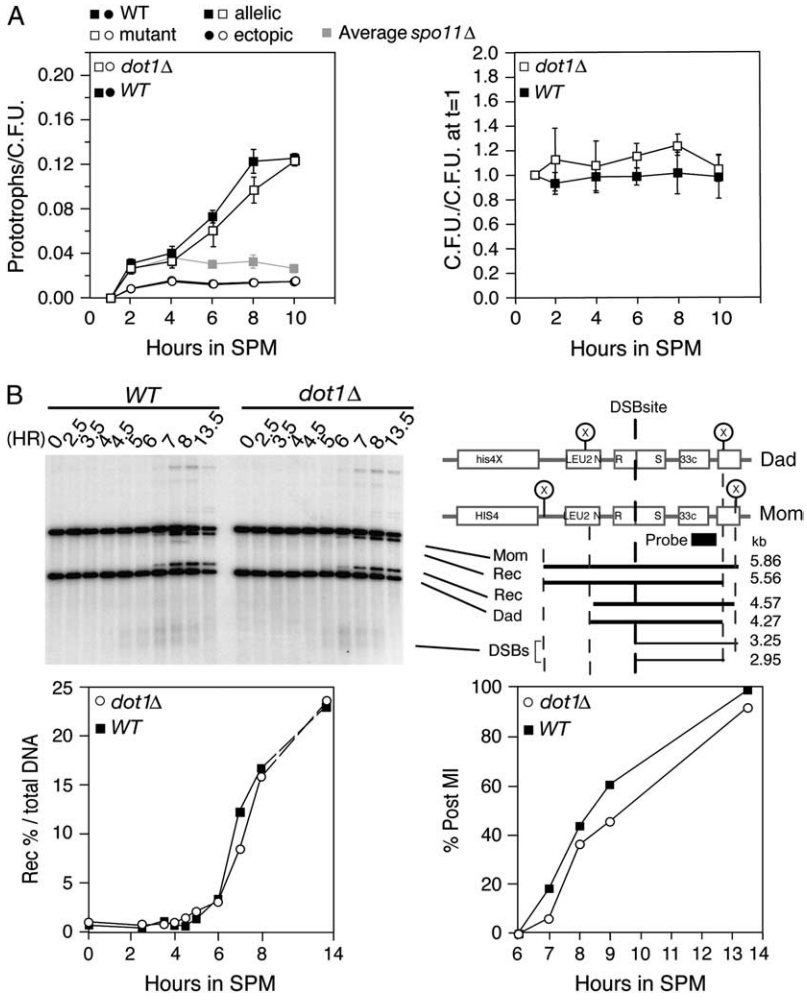

FIGURE 6.—Commitment to Cre-mediated *loxP* recombination and physical analysis of meiotic recombination at the *HIS4::LEU2* hotspot in wild-type and isogenic strains deleted for *DOT1*. (A) Allelic and ectopic collisions in wild-type (*ndt80*Δ) and the isogenic *dot1*Δ strains (left) with the return-to-growth viability for the time course (right). Graph parameters are the same as in Figure 1. (B) In a separate time course, recombinant formation at the *HIS4::LEU2* hotspot was analyzed by Southern blot (top) and bands corresponding to both recombinants in wild type (*NDT80*) (solid boxes) and *dot1*Δ (open circles) were quantified (bottom left). The timing of the meiosis I division (bottom right) was also determined in wild type (*NDT80*) (solid squares) and *dot1*Δ (open circles).

(B. SY and S. M. BURGESS, unpublished results) and others (TRELLES-STICKEN *et al.* 2005) have shown that microtubule-depolymerizing drugs do not appear to have an appreciable affect on DSB repair or on chromosome movement in budding yeast.

*dhc1*Δ*: DHC1*, also known as *DYN1*, encodes the heavy chain of dynein, a motor protein that participates in spindle positioning and anaphase chromosome segregation (SAUNDERS *et al.* 1995). In the fission yeast *S. pombe*, dynein is required for nuclear oscillations, meiotic chromosome pairing, recombination, and chromosome segregation in the presence or absence of recombination (DING *et al.* 2004; DAVIS and SMITH 2005).

We found that allelic and ectopic collisions in the *dhc1*Δ mutant were unchanged relative to the wild-type control (Figure 7A). RTG viability was not significantly altered in the *dhc1*Δ mutant (Figure 7B). The heavy chain of dynein, therefore, does not appear to play an appreciable role in CSHJ in *S. cerevisiae*, unlike its important role in *S. pombe* homolog pairing.

*mlp2*Δ*: MLP2* encodes a myosin-like protein associated with the Tel1-kinase pathway, which is involved in telomere-length control and localizes to the nuclear envelope (GALY *et al.* 2000; HEDIGER *et al.* 2002). Mlp2 also interacts with core components of the spindle-pole body (NIEPEL *et al.* 2005), where telomeres aggregate while in the bouquet (TRELLES-STICKEN *et al.* 2000). In mitotic division, *mlp2*Δ mutants display multiple aberrant microtubule organization centers (NIEPEL *et al.* 2005). However, telomeres still transiently cluster in the *mlp2*Δ mutant during prophase I (TRELLES-STICKEN *et al.* 2005).

In the *mlp2*Δ mutant, we observed unchanged allelic and ectopic collisions relative to the wild-type strain (Figure 7A). RTG viability was not affected in the *mlp2*Δ background (Figure 7B). Thus we found that *MLP2* plays no role in CSHJ or in the kinetics of prophase I progression.

Three levels of homologous chromosome interaction during meiosis in *S. cerevisiae* Our analysis has uncovered severe-to-moderate defects in achieving wild-type levels of meiotic homolog interactions (or CSHJ) among mutants affecting chromosome structure and homologous recombination. We summarize the results of our mutant analyses using the Cre/*loxP* collision assay from this work, PEOPLES *et al.* (2002), and PEOPLES-HOLST and BURGESS (2005) in Table 2. Cre/*loxP* phenotypes for all listed mutants were determined in the same genetic background using identical methodology.

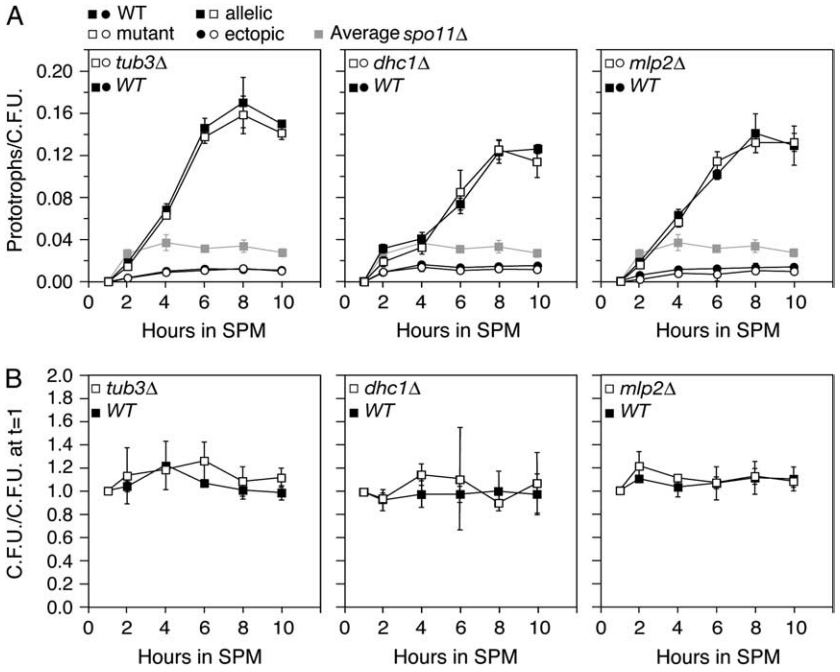

FIGURE 7.—Commitment to Cre-mediated *loxP* recombination in wild-type and isogenic strains deleted for *TUB3* (left), *DHC1* (middle), and *MLP2* (right). Graph parameters are the same as in Figure 1: (A) Allelic and ectopic collisions. (B) Return-to-growth viability for time courses in A.

Mutants fall roughly into three classes: (I) Severe CSHJ defects that reduce homolog associations to premeiotic levels were observed for mutations that eliminate entry into meiosis, meiotic DSB formation, or early stages of the meiotic recombination pathway up to the strand invasion step. (II) Intermediate CSHJ defects were found for mutations that specifically affect the efficiency or stability of joint molecules of meiotic recombination. (III) Minor or no CSHJ defects were conferred for mutations affecting late stages of meiotic recombination, the central element of the synaptonemal complex, or microtubule-mediated cellular movement.

The data presented above, along with that of many other investigators, provide a framework for understanding the high level of homologous chromosome interactions achieved during meiotic prophase I. Our current model elaborates on features described for three stages of homolog pairing observed cytologically in *Sordaria macrospora* (TESSE *et al.* 2003). From our results using the *in vivo* collision assay, we suggest that homologs achieve CSHJ in three steps:

1. "Somatic" homolog pairing: Homologous sites are loosely colocalized early in—and even prior to—meiosis by an unknown mechanism (WEINER and KLECKNER 1994; BURGESS *et al.* 1999); in all mutants evaluated thus far, none reduces the level of allelic collisions to ectopic levels (PEOPLES *et al.* 2002; PEOPLES-HOLST and BURGESS 2005; this work).
2. Coalignment by DSB-mediated homology search: Meiotic DSB formation at hundreds of sites throughout the genome initiates the Rad51/Dmc1-mediated homology search for homologous chromosome repair templates. Factors that elaborate the meiotic axial element function to bias the homology search away from sister chromatids by mediating the DSB-initiated homology search through the chromosomal axis (BLAT *et al.* 2002). The extended nucleoprotein filaments (predicted to be up to 1.5 times the length of B-form DNA, or around 10 times the length of chromatin-compacted DNA) could extend up to one-tenth the diameter of the nucleus (BURGESS 2002). These extensions could be thought of as fishing lines extending from the chromosomal axis. Line-like Rad51 foci have been observed in maize (FRANKLIN *et al.* 2003; PAWLOWSKI *et al.* 2003). In mice, Rad51 and Dmc1 foci have been detected along "axial bridges" that connect homologs prior to synapsis (TARSOUNAS *et al.* 1999). A homology search conducted by hundreds of meiotic DSBs throughout the genome would result in the formation of numerous unstable D-loop structures between a homolog pair. While these connections would contribute to the end-to-end pairing and coalignment of homologous chromosomes, they would be insufficient to achieve a fully juxtaposed state between homologs.
3. Stabilization and compaction of the strand invasion: Additional factors (such as *RDH54* and the *ZMM* group of genes) could promote the stabilization of strand-exchange intermediates by incorporating ssDNA from the extended nucleoprotein filament into chromatin by packaging DNA into the nucleosomes of the DSB repair substrate. By this mechanism, the two chromatids would essentially be "reeled" together from these points. Recombination events bound for a crossover fate via particularly stable joint molecules would make a special contribution *in cis* to tight associations between allelic sites and the progression of synapsis (J. C. MELL, B. L. WEINHOLZ and S. M.

TABLE 2

Summary of CSHJ phenotypes in mutants

Allele	CSHJ[a]	RTG[b]	Function of wild-type gene product in meiosis[c]
		DSB formation and axial element structure	
*spo11*Δ	I	+++	DSB formation; meiotic S-phase regulation
spo11-Y135F	I	+++	Spo11 catalytic residue
*rec104*Δ	I	+++	Meiotic DSB formation
*red1*Δ	I	+++	Axial element component; homolog partner choice
*hop1*Δ	I	+++	Axial element component; homolog partner choice
*mek1*Δ	I+	+++	Checkpoint control kinase; homolog partner choice
*hop2*Δ	I	+++	Homolog partner choice; interacts with Mnd1
*mnd1*Δ	I	++	Homolog partner choice; interacts with Hop2
		DSB processing and strand invasion	
*sae2*Δ	I+[d]	+	Removal of Spo11 from DSB; 5′-end resection of DSB
*rad55*Δ	I+	++[e]	Promotes Rad51–ssDNA filament formation
*dmc1*Δ	I	+++	RecA homolog; strand invasion; interacts with Rdh54
*rad51*Δ	I+	+	RecA homolog; strand invasion; interacts with Rad55
*rdh54*Δ	II	++	Swi/Snf homolog; interacts with Dmc1
*rad54*Δ	III	+++	Swi/Snf homolog; interacts with Rad51
		Stabilization of strand invasion and recombinant product formation	
*zip3*Δ	II	+++	Component of synapsis initiation complex; promotes CR formation
*mer3*Δ	II	+++	DNA helicase; promotes CR formation
*zip1*Δ	III−	+++	Transverse element of synaptonemal complex; promotes CR formation
*zip2*Δ	II	+++	Component of synapsis initiation complex; promotes CR formation
*ndj1*Δ	II	+++	Required for meiotic telomere reorganization and efficient recombination
*msh4*Δ	III	+++	MutS homolog; promotes CR formation; interacts with Msh5
*msh5*Δ	III	+++	MutS homolog; promotes CR formation; interacts with Msh4
*mus81*Δ	III	+++	Required for a subset of CR products arising via SDSA
		Additional genes	
*ime2*Δ	I[d]	+++	Entry into meiosis
*msc1*Δ	III	+++	Inhibits sister chromatid exchange?; genomic stability
*sgs1*Δ	II[d]	+++	RecQ helicase: suppressor of crossing over?
*srs2*Δ	III	+	DNA helicase; disrupts Rad51 presynaptic filament *in vitro*
*ris1*Δ	III−	+++	Antagonist of silencing; interacts with Dmc1
*dot1*Δ	III−	+++	Histone methytransferase; telomere position effect
*dhc1*Δ	III	+++	Dynein heavy chain; microtubule motor
*tub3*Δ	III	+++	α-Tubulin (partially redundant function with Tub1)
*mlp2*Δ	III	+++	Myosin-like protein; nuclear pore; telomere-length regulation

SDSA, synthesis-dependent strand annealing.

[a] Categories correspond to the level of allelic collisions observed: I, *spo11*Δ-like levels; II, intermediate levels; III, wild-type levels of allelic collision (CSHJ). "+" and "−" indicate slight increases or decreases from the category listed. Quantitative time courses for these single mutants are reported in this work, Peoples *et al.* (2002), and Peoples-Holst and Burgess (2005).

[b] Return-to-mitotic-growth phenotype: "+++" indicates wild-type levels of RTG viability; "++" indicates some defect (20–50% inviability by $t = 10$ hr); "+" indicates a relatively strong RTG defect of >50% inviability by $t = 10$ hr. These phenotypes may differ when compared to *NDT80* strains.

[c] References for functional roles are cited in this work, Peoples *et al.* (2002), and Peoples-Holst and Burgess (2005).

[d] Slight increase in ectopic collision levels.

[e] Transient RTG inviability.

Burgess, unpublished results). While the generation of fully repaired recombination products (crossovers in particular) would require additional factors (*e.g.*, *MSH4*) and the completion of chromosomal synapsis (*e.g.*, *ZIP1*), CSHJ would be essentially complete by pachytene.

This "fishing-line reeling" mechanism is reminiscent of recent work on DNA replication in *Escherichia coli*. In contrast to the traditional depiction of the DNA replication fork moving along DNA, the replisome remains relatively stable in the cell, while new copies of the chromosome are extruded from it and pass toward opposite poles (Gordon and Wright 1998). Similarly, instead of strand exchange simply increasing the size of the D-loop of two closely adjoined recombination partners, increasing the compaction and stability of joint molecules through the reestablishment of chromatin could be a

major driving force of homolog pairing during meiotic prophase I.

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