Developmental Cell, Vol. 5, 915–925, December, 2003, Copyright ©2003 by Cell Press

The Importance of Genetic Recombination for Fidelity of Chromosome Pairing in Meiosis

Hideo Tsubouchi[1] and G. Shirleen Roeder[1,2,*]
[1]Howard Hughes Medical Institute
Department of Molecular, Cellular,
and Developmental Biology
[2]Department of Genetics
Yale University
New Haven, Connecticut 06520

Summary

In budding yeast, absence of the Hop2 protein leads to extensive synaptonemal complex (SC) formation between nonhomologous chromosomes, suggesting a crucial role for Hop2 in the proper alignment of homologous chromosomes during meiotic prophase. Genetic analysis indicates that Hop2 acts in the same pathway as the Rad51 and Dmc1 proteins, two homologs of *E. coli* RecA. Thus, the *hop2* mutant phenotype demonstrates the importance of the recombination machinery in promoting accurate chromosome pairing. We propose that the Dmc1/Rad51 recombinases require Hop2 to distinguish homologous from nonhomologous sequences during the homology search process. Thus, when Hop2 is absent, interactions between nonhomologous sequences become inappropriately stabilized and can initiate SC formation. Overexpression of *RAD51* largely suppresses the meiotic defects of the *dmc1* and *hop2* mutants. We conclude that Rad51 is capable of carrying out a homology search independently, whereas Dmc1 requires additional factors such as Hop2.

Introduction

Diploid eukaryotes produce haploid gametes through meiosis. During meiosis, a single round of DNA replication is followed by two successive rounds of nuclear division, meiosis I and meiosis II. Homologous chromosomes segregate at meiosis I, and sister chromatids separate at meiosis II. During prophase of meiosis I, homologs recognize each other and undergo high levels of genetic recombination. Reciprocal recombination is crucial for the formation of chiasmata, which are physical connections between homologs that ensure their correct segregation at the first meiotic division.

The molecular mechanism of meiotic recombination has been well studied in budding yeast. The process starts with double-strand breaks (DSBs) formed by the Spo11 protein, a type II topoisomerase homolog (Keeney, 2001). The DSB ends are degraded from their 5′ ends, giving rise to single-strand DNA (ssDNA) (Sun et al., 1991; Bishop et al., 1992). This ssDNA is thought to be used for homology searching by strand exchange enzymes (recombinases). Eventually, the ssDNA invades homologous sequences in a nonsister chromatid, giving rise to a single-end invasion intermediate and/or a double-Holliday junction (Schwacha and Kleckner, 1994; Hunter and Kleckner, 2001). It has been proposed that crossovers are formed mainly by resolution of double-Holliday junctions (Allers and Lichten, 2001).

Budding yeast has two major recombinases, Rad51 and Dmc1, both of which are homologs of the bacterial RecA protein (Bishop et al., 1992; Shinohara et al., 1992). Rad51 is involved in both mitotic and meiotic recombination, whereas Dmc1 is a meiosis-specific protein involved only in meiotic recombination. Rad51 forms a nucleoprotein filament (Ogawa et al., 1993) and catalyzes pairing and exchange of strands between homologous DNA molecules (Sung, 1994). The Dmc1 protein promotes renaturation of complementary ssDNA and assimilation of homologous ssDNA into duplex DNA (Hong et al., 2001). In the absence of these proteins, meiotic recombination is severely reduced or abolished, and the meiotic cell cycle is delayed (or arrested) because of the persistence of recombination intermediates.

Rad51 and Dmc1 appear to perform overlapping as well as distinct functions in meiotic recombination (Dresser et al., 1997; Shinohara et al., 1997). Rad51 and Dmc1 colocalize to a number of foci on meiotic chromosomes during leptotene and zygotene (Bishop, 1994). Although a large fraction of these foci overlap, there is a subset of Rad51 foci and Dmc1 foci that do not overlap (Dresser et al., 1997; Shinohara et al., 2000). The localization of Rad51 on meiotic chromosomes is independent of Dmc1, whereas that of Dmc1 is largely dependent on Rad51 (Bishop, 1994).

Meiotic recombination is concurrent with the development of synaptonemal complex (SC) (reviewed by Roeder [1997]; Zickler and Kleckner [1999]). In preparation for SC formation, two sister chromatids of a single chromosome develop a common proteinaceous core, called an axial element. Within the context of SC, axial elements are referred to as lateral elements. Two lateral elements representing homologs are connected to each other along their entire length by the central region of the SC. In budding yeast, the Zip1 protein is the major component of the central region (Sym et al., 1993). In the absence of Zip1, homologs are aligned side by side and intimately connected to each other at a few points, called axial associations. In the absence of Rad51 or Dmc1, axial associations are not formed, and homolog pairing is reduced, suggesting that an axial association reflects a recombination intermediate (Rockmill et al., 995). Two proteins, Zip2 and Zip3, localize to axial associations, where they promote polymerization of the Zip1 protein (Chua and Roeder, 1998; Agarwal and Roeder, 2000). Zip2 and Zip3 colocalize, and Zip3 interacts with various recombination proteins (e.g., Rad51, Rad57, Mre11, Msh4, and Msh5), suggesting that SC formation initiates at sites where recombination is taking place.

Previous studies showed that a complex consisting of the Hop2 and Mnd1 proteins plays an essential role during meiosis (Leu et al., 1998; Rabitsch et al., 2001; Tsubouchi and Roeder, 2002; Gerton and DeRisi, 2002). In the absence of these proteins, the cell cycle arrests

*Correspondence: shirleen.roeder@yale.edu

at the pachytene stage of meiotic prophase. DSBs are not repaired, and recombinases accumulate on meiotic chromosomes to aberrantly high levels (Leu et al., 1998; Tsubouchi and Roeder, 2002). Homolog pairing is substantially reduced, and extensive synapsis occurs between nonhomologous chromosomes (Leu et al., 1998). This phenotype suggests that the Hop2/Mnd1 complex plays a role in homology searching and/or recognition.

In budding yeast, genetic studies have shown that the meiotic recombination machinery has the capacity to detect homology between sequences at ectopic locations almost as efficiently as allelic locations (Jinks-Robertson and Petes, 1985; Lichten et al., 1987; Haber et al., 1991). This observation demonstrates that recombination-mediated homology searching occurs throughout the entire genome. On the other hand, fluorescence in situ hybridization (FISH) has demonstrated that some homolog pairing occurs even in the absence of recombination (Loidl et al., 1994; Weiner and Kleckner, 1994; Nag et al., 1995). Thus, meiotic recombination and recombination-independent pairing mechanisms seem to function redundantly in homolog alignment. However, the relative contribution of each mechanism to homolog alignment remains obscure.

In this study, we provide evidence that Hop2 is involved in meiotic recombination. Epistasis analysis indicates that Hop2 acts in the same pathway as Rad51 and Dmc1. Thus, the nonhomologous synapsis observed in the *hop2* mutant demonstrates the importance of the recombination machinery in homolog alignment. We propose that Hop2 executes a novel step in meiotic recombination, downstream of Dmc1 and Rad51, and this step ensures accurate and efficient homology searching. In addition, we have found that overproduction of Rad51 suppresses the defect in meiotic recombination in *dmc1*, and this suppression does not require the Hop2 protein. We propose that there are two pathways for recombination-mediated homolog pairing. One pathway relies on Rad51 but not Dmc1 or Hop2/Mnd1; the other utilizes Dmc1 and the Hop2/Mnd1 complex (and probably also Rad51).

Results

A *hop2* Haploid Fails to Repair Meiotic DSBs

Haploid yeast strains initiate meiotic recombination when introduced into sporulation medium but are prevented from repairing DSBs using information from homologous chromosomes. DSB repair is therefore delayed, but DSBs are eventually repaired by recombination between sister chromatids (de Massy et al., 1994; Gilbertson and Stahl, 1994). If the *hop2* defect in DSB repair is indirectly caused by a defect in pairing, then wild-type and *hop2* haploid cells should show similar defects in DSB repair. On the other hand, if *hop2* is defective in recombination, then the defect in DSB repair in a *hop2* haploid should be more severe than that of wild-type. In this case, the phenotype would be the sum of the defect in pairing (due to the absence of homologs) plus the defect in recombination (due to the *hop2* mutation).

DSB repair was monitored in haploid strains carrying both *MAT***a** and *MAT*α. DSBs generated on chromosome III were detected by Southern blot analysis following the separation of chromosomes by pulsed-field gel electrophoresis. In wild-type, DSBs appear and then gradually disappear (Figure 1A). On the other hand, DSBs persist in *hop2* haploid cells past the time when DSBs are repaired in wild-type (Figure 1A), similar to the *hop2* mutant diploid (Leu et al., 1998). DSBs also accumulate in a haploid *dmc1* mutant (Figure 1A). These results indicate that the *hop2* defect in DSB repair is more severe than the defect caused merely by the absence of homolog pairing. The defect is better explained if the *hop2* mutant is defective in meiotic recombination per se, just like *dmc1*.

Mutations in *RAD51* and *DMC1* Suppress the Homolog Pairing Defect and the Formation of Polycomplexes in *hop2*

The Rad51 and Dmc1 proteins accumulate to aberrantly high levels on chromosomes in *hop2* cells during meiotic prophase (Leu et al., 1998). It is possible that the excessive recombination proteins are acting aberrantly in *hop2* (i.e., promoting interactions between nonhomologous chromosomes), thus reducing homolog pairing. If this is the case, then removing these proteins from a *hop2* mutant should improve homolog pairing. To test this possibility, homolog pairing was assayed by FISH, using DNA probes specific for chromosomes III and V to analyze spread meiotic nuclei. To obtain meiotic cells from the pachytene stage, when pairing is maximal, the *ndt80* mutant was used. Ndt80 is a global activator of a large set of sporulation-specific genes (Chu and Herskowitz, 1998); in its absence, the meiotic cell cycle arrests at the pachytene stage.

Cells from the *ndt80* mutant show nearly complete homolog pairing at the time point examined (24 hr after the introduction into sporulation medium) (Figures 2A and 2B). In the *hop2* mutant, homolog pairing is substantially reduced (Figures 2A and 2B). In the absence of Rad51 or Dmc1, however, homolog pairing is less severely impaired (Figures 2A and 2B). The frequency of pairing in the *dmc1 rad51* double mutant is similar to the corresponding single mutants (Figure 2B). When one or both of the *RAD51* and *DMC1* genes are disrupted in *hop2*, homolog pairing occurs at higher than the *hop2* level and approximately at the level of the *rad51* or *dmc1* mutant (Figures 2A and 2B). Suppression of the *hop2* pairing defect by mutation of *RAD51* or *DMC1* indicates that the reduction in homolog pairing in *hop2* is at least partially attributable to the activity of Rad51 and Dmc1.

Nuclei from the *hop2* mutant frequently contain a polycomplex (Figure 3A), which is an aggregate of SC proteins unassociated with chromatin (Loidl et al., 1994). Polycomplex formation is due to reduced efficiency of loading of Zip1 onto chromosomes and can result from a defect in homolog pairing (Loidl et al., 1994). In the wild-type strain used in this study, the Zip1 protein fully extends along chromosomes (Figure 3A), and very few nuclei contain a polycomplex (Figures 2C and 2D). In the *rad51* and *dmc1* mutants, Zip1 staining tends to be mostly linear, and the frequencies of polycomplex formation are slightly higher than in wild-type (Figures 2C, 2D, and 3A). In *hop2*, chromosomes tend to be less synapsed, and the frequency of polycomplex formation

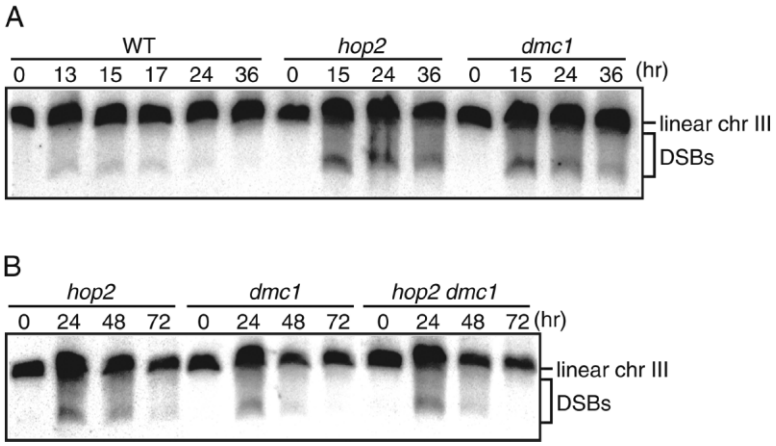

Figure 1. DSBs Are Not Repaired in a *hop2* Haploid but Are Repaired in a *hop2 dmc1* Diploid

Cells 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). Strains used in (A) are wild-type (WT), *hop2::ADE2*, and *dmc1::URA3* haploids carrying both *MAT***a** and *MAT*α. Strains used in (B) are diploids homozygous for *hop2::ADE2*, *dmc1::KAN*, or *hop2::ADE2 dmc1::KAN*. The positions of linear chromosome III and molecules that have sustained one or more DSBs are indicated to the right of the gel.

is much higher (Figures 2C, 2D, and 3A). Consistent with the improvement in homolog pairing, a mutation in *RAD51* or *DMC1* substantially extends synapsis and reduces polycomplex formation in *hop2* (Figures 2C, 2D, and 3A). These data provide further support for the proposal that mutations in *RAD51* and *DMC1* suppress the *hop2* defect in homolog pairing.

These analyses of homolog pairing and polycomplex formation show that *dmc1* and *rad51* are epistatic to *hop2*, suggesting that Dmc1, Rad51, and Hop2 act in the same pathway, with Hop2 acting downstream of Dmc1/Rad51.

The *dmc1* Mutation Bypasses Cell Cycle Arrest in *hop2*

The *hop2* mutant sporulates very poorly compared to the *dmc1* and *rad51* mutants (Figure 4A). The *hop2 dmc1* double mutant shows a level of sporulation similar to *dmc1* (Figure 4A), indicating that *dmc1* is epistatic to *hop2* for sporulation. In addition, the *dmc1* and *hop2 dmc1* mutants show the same level of spore viability (Figure 4B). If DSBs remain unrepaired in the double mutant, then spore viability should be further reduced compared to the *dmc1* single mutant. Thus, the suppression of *hop2* arrest by *dmc1* cannot be attributed simply to a defective checkpoint mechanism. The *dmc1* mutation must somehow allow DSB repair in the absence of Hop2. To test this possibility directly, DSBs generated on chromosome III were monitored as described above. In the *hop2* mutant, DSBs persist past the time when DSBs disappear in *dmc1* (Figure 1B). In *hop2 dmc1*, DSBs disappear with kinetics similar to *dmc1* (Figure 1B), indicating that DSBs are repaired in the *hop2 dmc1* double mutant. Taken together, these results indicate that meiotic arrest of *hop2* is due to the action of the Dmc1 protein.

In contrast to the situation with *dmc1*, the *rad51 hop2* mutant sporulates as poorly as the *hop2* mutant (Figure 4A). Thus, Rad51 is not responsible for cell cycle arrest in *hop2*.

Overproduction of Rad51 Suppresses Both the Sporulation Defect and the Spore Inviability of *dmc1* and *hop2* Strains

The *MND1* gene was previously identified as a multicopy suppressor of the spore inviability phenotype of a *hop2* temperature-sensitive mutant (Tsubouchi and Roeder, 2002). The same screen also identified the *RAD51* gene. However, unlike *MND1*, overproduction of Rad51 also suppresses the *hop2* null mutant. In an effort to increase the efficiency of suppression, the *RAD51* gene was cloned into a vector, YEpFAT4, that is maintained at a higher copy number than standard multicopy vectors (Conrad et al., 1990). Overexpression of *RAD51* in YEpFAT4 suppresses the sporulation defect and spore inviability of both *hop2* and *mnd1* (Figures 4C and 4D).

Epistasis analysis indicates that Hop2 and Dmc1 act in the same pathway (Figures 4A and 4B); thus, *dmc1* was also tested for suppression by Rad51 overproduction. Remarkably, sporulation and spore viability in *dmc1* are restored to nearly wild-type levels by Rad51 overproduction (Figures 4C and 4D). Furthermore, *dmc1 hop2* strains overproducing Rad51 behave similarly to *dmc1* strains overproducing Rad51 (Figures 4C and 4D). In contrast, Dmc1 overproduction does not improve sporulation in the *rad51* mutant; instead, excess Dmc1 causes cell cycle arrest in *rad51* (Figure 4C).

Overproduction of Rad51 Restores Crossing Over in the *dmc1* Mutant

In the *dmc1* mutant, crossing over is reduced more than 4-fold compared to wild-type (Rockmill et al., 1995). In contrast, the *dmc1* mutant overproducing Rad51 shows $\sim$80% spore viability, suggesting that the level of crossing over is nearly wild-type. To test this possibility, crossover frequencies in a *dmc1* strain overproducing Rad51 were measured in four intervals by tetrad analysis. In all intervals examined, 70%–90% of the wild-type level of crossing over was observed (Figure 5A), indicating that overproduction of Rad51 increases crossing over in the *dmc1* mutant.

Crossovers are not distributed along chromosomes at random in wild-type. Instead, adjacent crossovers rarely occur in close proximity to each other. This phenomenon, known as crossover interference, was measured by calculating the NPD ratio, which can be loosely defined as the frequency of double crossovers observed in a marked interval divided by the frequency expected (see legend to Table 1 for formula) (Chua and Roeder, 1997). An NPD ratio of less than 1 is indicative of positive

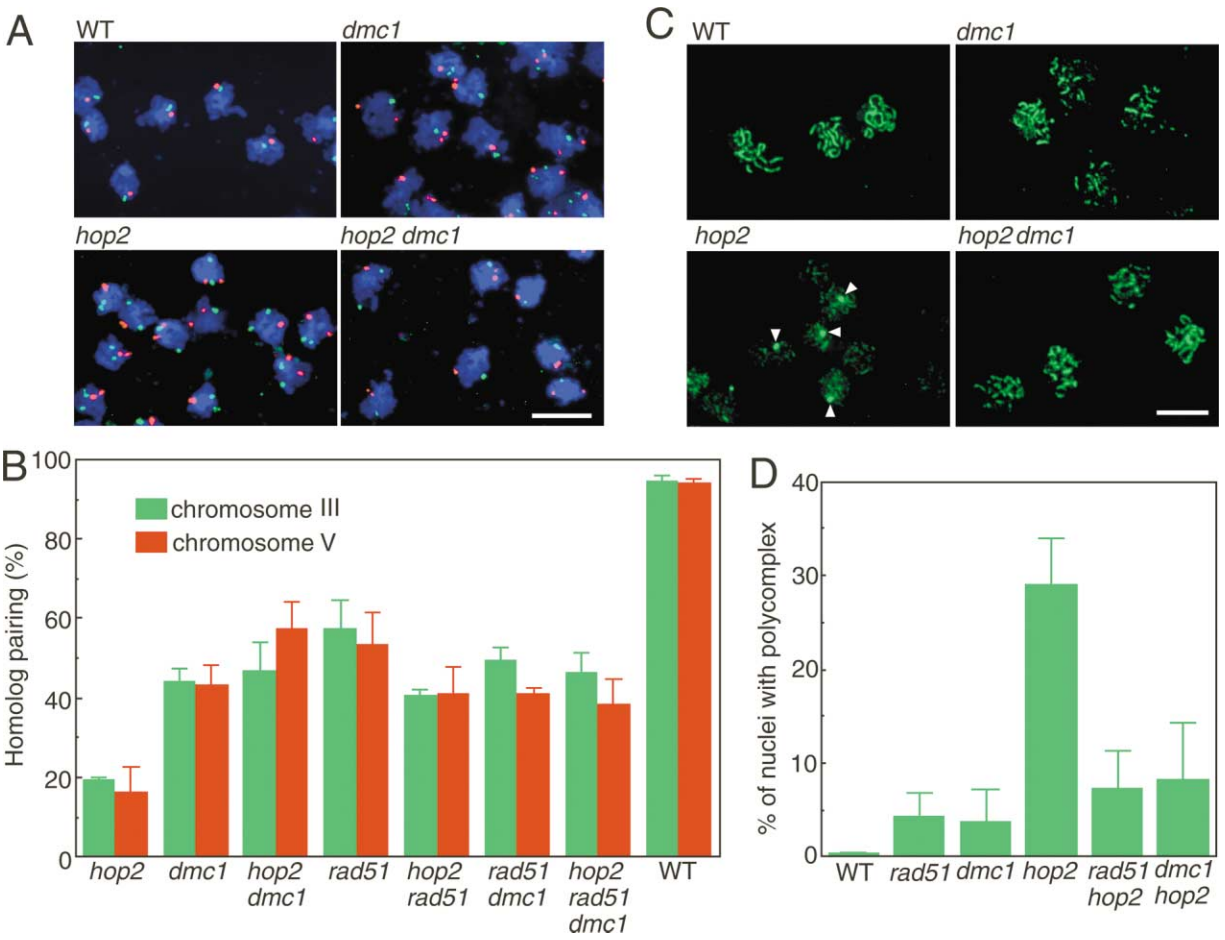

Figure 2. The *dmc1* and *rad51* Mutations Partially Improve Homolog Pairing and Suppress Polycomplex Formation in *hop2*

(A) Typical images of FISH analysis. Meiotic chromosomes were surface spread and hybridized with probes specific for chromosome III (green) and V (red). Nuclei were also stained with the DNA binding dye DAPI (4′,6′-diamidino-2-phenylindole) (blue). Scale bar, 10 μm.

(B) Quantitative analysis of homolog pairing. For each strain, FISH analysis was carried out in two or more independent experiments, with at least 140 nuclei scored in each experiment. Shown is the frequency of pairing for chromosomes III and V. Pairing was measured after 24 hr of sporulation, when wild-type cells show more than 90% pairing. Chromosomes were classified as unpaired if there were two separate signals of the same color in a single spread nucleus. Chromosomes were classified as paired if their FISH signals touched each other or overlapped. Error bars represent standard deviations.

(C) Examples of spread nuclei stained with antibodies to Zip1. The white arrowheads indicate polycomplexes. Scale bar, 8 μm.

(D) Quantitative analysis of polycomplex formation. For each strain, the fraction of nuclei carrying polycomplexes was measured in two or more independent experiments, with at least 200 nuclei scored in each experiment. Chromosome spreads were prepared after 24 hr of sporulation. Error bars represent standard deviations.

Strains analyzed are *hop2::ADE2*, *dmc1::URA3*, *hop2::ADE2 dmc1::URA3*, *rad51::URA3*, *hop2::ADE2 rad51::URA3*, *rad51::URA3 dmc1::URA3*, *hop2::ADE2 rad51::URA3 dmc1::URA3*, and wild-type (WT); all strains are homozygous for *ndt80::LEU2*.

crossover interference. In two intervals examined, *HIS4-CEN3* and *CEN3-MAT*, crossover interference is observed in *dmc1* overproducing Rad51, but interference is reduced compared to wild-type (Table 1). In the *MAT-RAD18* interval, the same level of crossover interference is observed both in wild-type and in *dmc1* overproducing Rad51 (Table 1). Thus, crossover interference does operate in *dmc1* cells overproducing Rad51, although interference is reduced in some intervals.

Msh4 is a meiosis-specific protein required for the wild-type level of crossing over and thus for normal spore viability (Ross-Macdonald and Roeder, 1994). To assess the importance of Msh4 in *dmc1* strains overproducing Rad51, spore viability was assessed. In the *dmc1 msh4* double mutant overproducing Rad51, spore viability is approximately half that of the *dmc1* mutant overproducing Rad51 (Figure 5B). Thus, spore viability in *dmc1* overproducing Rad51 is partially dependent on Msh4, just as in wild-type. There is no major effect of Rad51 overproduction on spore viability in the *msh4* mutant (Figure 5B).

The formation of axial associations depends on recombination proteins; therefore, these cytological structures probably reflect recombination intermediates (Rockmill et al., 1995). To test if overproduction of Rad51 restores axial associations in the *dmc1 zip1* mutant,

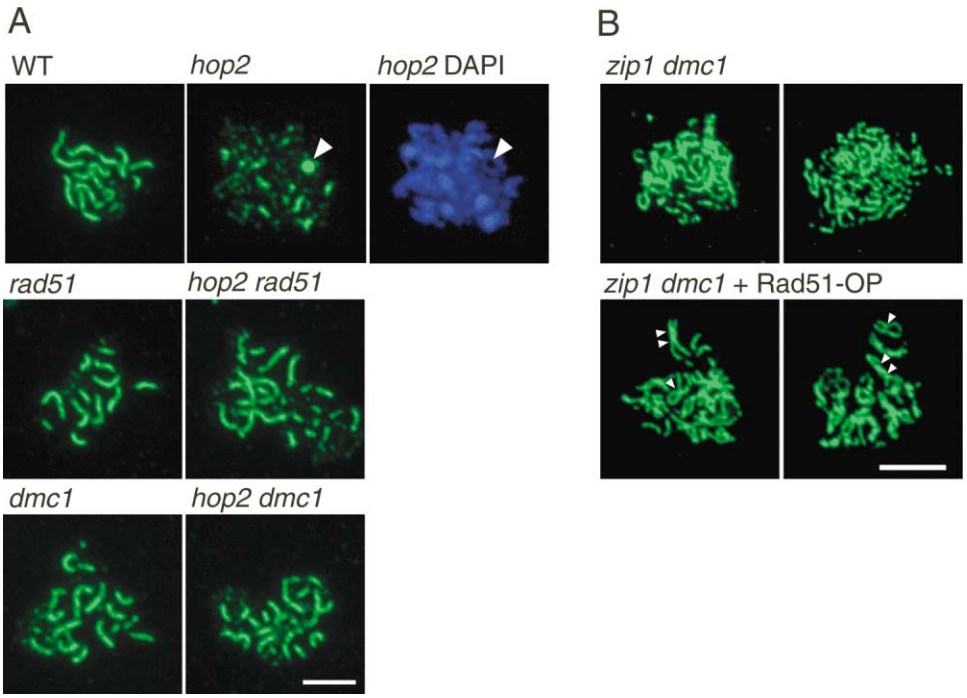

Figure 3. Suppression of the *hop2* Defects in Meiotic Chromosome Morphogenesis by *dmc1/rad51* Mutation and Rad51 Overproduction

(A) Examples of nuclei stained with antibodies to Zip1 (green). A nucleus from *hop2* was also stained with DAPI (blue). The white arrowhead indicates the location of a polycomplex. Scale bar, 4 μm. Strains analyzed are *hop2::ADE2*, *dmc1::URA3*, *hop2::ADE2 dmc1::URA3*, *rad51::URA3*, *hop2::ADE2 rad51::URA3*, and wild-type (WT); all strains are homozygous for *ndt80::LEU2*.

(B) Chromosome axes were visualized by staining spread chromosomes with antibodies to the Red1 protein (green). Chromosome spreads were prepared at 20 hr of sporulation. The white arrowheads indicate the positions of axial associations. Scale bar, 5 μm. Strains shown are carrying a multicopy vector YEpFAT4 containing no insert or *RAD51*; only those carrying *RAD51* are indicated as + Rad51-OP. Strains analyzed are *dmc1::KAN zip1::LYS2* + YEpFAT4 and *dmc1::KAN zip1::LYS2* + YEpFAT4-*RAD51*.

meiotic chromosomes were spread, and the Red1 protein was stained to visualize chromosome axes. Remarkably, nearly half of all spreads exhibited a number of well-defined axial associations, whereas few, if any, axial associations were observed in the *dmc1 zip1* double mutant (Rockmill et al., 1995) (Figures 3B and 5C).

Taken together, these results suggest that recombination in *dmc1* strains overproducing Rad51 is mechanistically similar to that in wild-type (see Discussion).

Discussion

Genetic Recombination Plays an Important Role in Aligning Homologous Chromosomes

Two pathways for homolog pairing are believed to operate in budding yeast (see Introduction). One pathway relies on homology searching mediated by recombination enzymes and presumably is responsible for ectopic recombination events. The other pathway is independent of recombination; although the molecular mechanism remains unknown, it is thought that telomere clustering plays an important role (Rockmill and Roeder, 1998; Trelles-Sticken et al., 1998, 2000). For technical reasons, it has been difficult to assess the relative importance of the two pairing mechanisms in meiosis. FISH detects low levels of pairing even in mutants completely defective in the initiation of meiotic recombination (Loidl et al., 1994; Weiner and Kleckner, 1994; Nag et al., 1995; Peoples et al., 2002). However, this method involves surface spreading of meiotic chromosomes, and weak interactions between homologs could be destabilized during this procedure (see Nag et al. [1995] for discussion). Thus, it is unclear the extent to which FISH underestimates the contribution of the recombination-independent pairing pathway. In principle, all pairing might be independent of recombination, and recombination might serve only to stabilize otherwise weak interhomolog interactions.

Our results demonstrate that Hop2 acts in the same pathway as Dmc1 and Rad51 and thus participates in the process of meiotic recombination. The phenotype of *hop2* therefore provides a measure of the importance of the recombination machinery in the alignment of homologous chromosomes. In the absence of Hop2, chromosome III is paired in only ~20% of nuclei; a similar efficiency of pairing is seen for chromosome V. This level of pairing is similar to what is seen in mutants that fail to make meiotic DSBs (Nag et al., 1995). In *hop2*, however, the reduction in pairing cannot be attributed to a failure to stabilize pairing interactions. Extensive stable interactions (i.e., SC) are formed both between homologs and between nonhomologous chromosomal regions. SC formation between different chromosomes results in an extensive branched network, such that individual chromosomes are not easily separated during

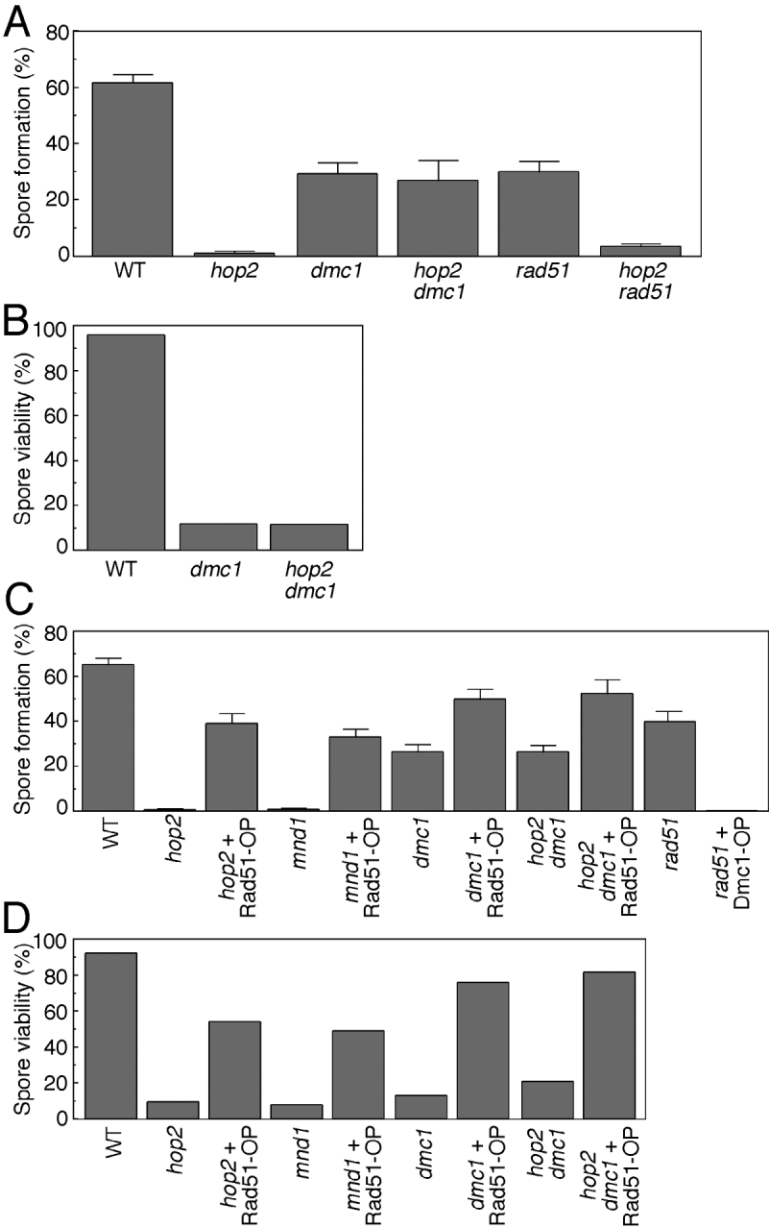

Figure 4. Suppression of the *hop2* Sporulation Defect by *dmc1* Mutation and Rad51 Overproduction

(A) Cells were sporulated at 30°C for 2 days; spore formation was measured as described in Experimental Procedures. Error bars represent standard deviations.

(B) To measure spore viability, 44 tetrads for wild-type and 88 tetrads each for the *dmc1* and *hop2 dmc1* mutants were dissected. Strains analyzed in (A) and (B) are wild-type (WT), *hop2::ADE2*, *dmc1::URA3*, *hop2::ADE2 dmc1::URA3*, *rad51::URA3* and *hop2::ADE2 rad51::URA3*.

(C) Cells were sporulated at 30°C for 3 days; spore formation was then measured as described in Experimental Procedures. Error bars represent standard deviations.

(D) To measure spore viability, 110 tetrads were dissected for *hop2* + Rad51-OP and *mnd1* + Rad51-OP, 66 tetrads each for WT, *hop2* and *mnd1*, and 44 tetrads for each of the remaining strains. Strains shown carry a multicopy vector YEpFAT4 containing no insert, *RAD51*, or *DMC1*; only those carrying a vector with an insert are indicated as + Rad51-OP or + Dmc1-OP. OP, overproduction. Strains analyzed in (C) and (D) are *hop2::ADE2* + YCp-*HOP2* (wild-type [WT]), *hop2::ADE2* + YEpFAT4, *hop2::ADE2* + YEpFAT4-*RAD51*, *mnd1::KAN* + YEpFAT4, *mnd1::KAN* + YEpFAT4-*RAD51, dmc1::KAN* + YEpFAT4, *dmc1::KAN* + YEpFAT4-*RAD51, hop2::ADE2 dmc1::KAN* + YEpFAT4, *hop2::ADE2 dmc1::KAN* + YEpFAT4-*RAD51*, *rad51::hisG* + YEpFAT4-*DMC1*, and *rad51::hisG* + YEpFAT4.

spreading (Leu et al., 1998). Some chromosomes are folded back on themselves due to synapsis between different parts of the same chromosome. Thus, in the absence of an effective recombination-mediated homology search, interactions take place between nonhomologous chromosomal segments, and these are stabilized by SC formation. Leu et al. (1998) estimated that greater than 50% of chromosomes III are engaged in nonhomologous synapsis in the absence of Hop2.

In the *hop2* Mutant, Rad51 and Dmc1 Inhibit Recombination and Promote Associations between Nonhomologous Chromosomes

Genetic analyses show that Hop2, Dmc1, and Rad51 belong to the same epistasis group with respect to homolog pairing and SC formation, indicating that these proteins work in the same recombination pathway. Assuming that the recombination pathway consists of a linear sequence of dependent events, Hop2 works downstream of Dmc1/Rad51. This view is consistent with the observation that the Dmc1 and Rad51 proteins accumulate on chromosomes in the *hop2* mutant.

What do the Dmc1 and Rad51 proteins do in the absence of Hop2? Dmc1 and Rad51 are presumably recruited to DSB sites; in the absence of Hop2, the amount of Dmc1 and Rad51 on meiotic chromosomes increases over time. Apparently, both DSBs and foci of Dmc1 and Rad51 persist. We propose that, in the absence of Hop2, Dmc1 is not just nonfunctional but is inhibitory to meiotic cell cycle progression. One possibility for this inhibitory effect is that the persistent Dmc1 protein prevents other recombination enzymes from acting on DSBs. If persistent Dmc1 prevents Rad51 from acting, thus inhibiting

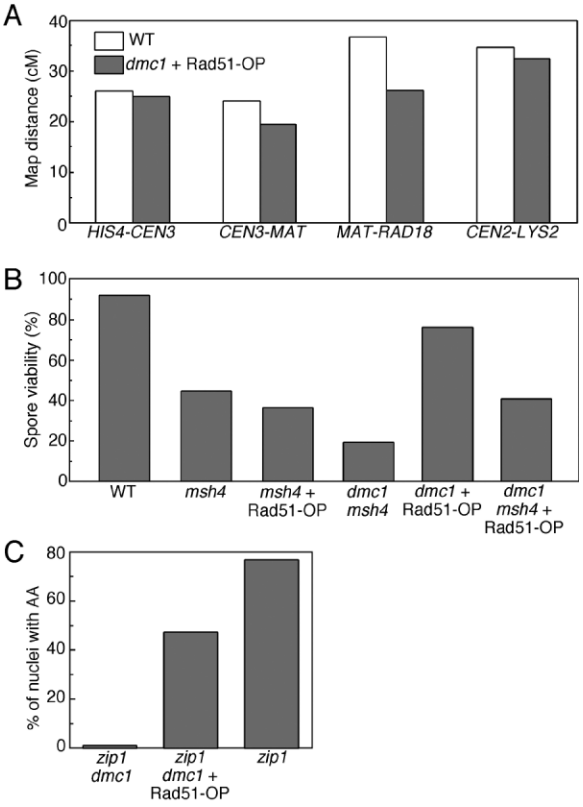

Figure 5. The *dmc1* Mutant Overproducing Rad51 Behaves like Wild-Type with Respect to Recombination

(A) Crossing over was measured as described previously (Chua and Roeder, 1997). Strains analyzed are *dmc1::KAN* + YCp-*DMC1* (wild-type [WT]) and *dmc1::KAN* + YEpFAT4-*RAD51*; see Experimental Procedures for marker information.

(B) To measure spore viability, 66 tetrads were dissected for each strain. Strains shown carry a multicopy vector YEpFAT4 containing no insert or *RAD51*; only those strains carrying a vector with an insert are indicated as + Rad51-OP. OP, overproduction. Strains analyzed are wild-type + YEpFAT4 (WT), *msh4::ADE2* + YEpFAT4, *msh4::ADE2* + YEpFAT4-*RAD51*, *dmc1::KAN msh4::ADE2* + YEpFAT4, *dmc1::KAN* + YEpFAT4-*RAD51*, and *dmc1::KAN msh4::ADE2* + YEpFAT4-*RAD51*.

(C) Quantitative analysis of axial associations (AA). The fraction of nucleoids having at least one well-defined axial association was determined for each strain. Due to difficulties in quantitating axial associations, no attempt was made to determine the number of associations per nucleus. Strains shown carry a multicopy vector YEpFAT4 containing no insert or *RAD51*; only those carrying *RAD51* are indicated as + Rad51-OP. Strains analyzed are *dmc1::KAN zip1::LYS2* + YEpFAT4, *dmc1::KAN zip1::LYS2* + YEpFAT4-*RAD51*, and *zip1::LYS2* + YEpFAT4.

DSB repair, then cell cycle progression would be arrested.

Another possibility for the inhibitory effect is that the *hop2* defect in homolog pairing is due to Dmc1 and Rad51 promoting (or stabilizing) interactions between nonhomologous chromosomes during the homology search process. FISH analysis indicates that pairing in *hop2* is improved by a *dmc1* or *rad51* mutation (and therefore presumably nonhomologous synapsis is decreased); thus, Dmc1 and Rad51 are responsible for associating nonhomologous chromosomes. This raises the possibility that Dmc1 and Rad51, in the absence of Hop2, form aberrant homology search intermediates between nonhomologous sequences. These two possibilities, an inhibitory effect of Dmc1 on recombination and formation of aberrant homology search intermediates by Dmc1/Rad51, are not mutually exclusive. In both cases, recombination intermediates would persist, thus activating the pachytene checkpoint pathway (Bailis and Roeder, 2000) and leading to cell cycle arrest.

A third possibility is that excess Dmc1 and Rad51 promote promiscuous loading of Zip1 onto chromosomes in the *hop2* mutant, such that Zip1 then randomly captures nearby chromosome axes. Such capture might impose spatial constraints on chromosomes that effectively prevent homologous sequences from accessing each other. In this case, a *zip1* mutation should improve homolog pairing in *hop2*. However, a *hop2 zip1* double mutant shows a level of homolog pairing similar to the *hop2* single mutant (data not shown), arguing against a role for Zip1 in promoting nonhomologous interactions.

What Is the Molecular Function of the Hop2 Protein?

We propose that nonhomologous synapsis in *hop2* reflects a defect in a Hop2-dependent step in meiotic recombination that follows DSB formation and resection. We suppose that recombining DNA molecules achieve homologous alignment through the following steps (Figure 6A). The first step is a recombinase-mediated homology search between the exposed ends and sequences anywhere in the genome that recombination proteins can access (Figure 6Ai). The second step would be an assessment of homology for each pair of interacting molecules (Figure 6Aii). The next step could be either of the following two possibilities, depending on the outcome of the homology assessment. If the two tethered sequences are perceived as "different," then they would dissociate, and the homology search process would start over again (Figure 6Aiii). If the sequences are found to be "similar," then they would commit to recombination and form a recombination intermediate (Figure 6Aiv).

Based on this simple model, there are two possible steps for Hop2 action. The first possibility is that Hop2 plays a role in deciding whether sequences are similar (Figure 6Aii), perhaps by facilitating recombinase activity. In the *hop2* mutant, the Dmc1 and Rad51 proteins are not dissuaded from promoting an interaction when two sequences are dissimilar, and these sequences then become inappropriately stably paired. These intermediates of homology search may be similar to structures formed in vitro by RecA and HsRad51 (Tsang et al., 1985; Baumann et al., 1996). RecA and HsRad51 form aggregates of ssDNA and duplex DNA, regardless of homology; and these aggregates are presumed to represent intermediates in the homology search process.

A second possibility for Hop2 function is in promoting the dissociation of two nonhomologous sequences (Figure 6Aiii). After the homology search deems the sequences dissimilar, the DSB end must dissociate from the intact duplex and be available to reinitiate a homology search. If this dissociation step fails, the nonhomologous sequences will remain associated, thus impeding homologous pairing. This function could be performed

Table 1. Crossover Interference

Strain	Interval	PD	TT	NPD	NPDexp	NPD Ratio	p Value
Wild-type	*HIS4-CEN3*	245	232	3	22.5	0.13	<0.0001
	CEN3-MAT	266	215	3	18.1	0.17	0.0004
	MAT-RAD18	179	293	10	46.8	0.21	<0.0001
dmc1 + Rad51-OP	*HIS4-CEN3*	242	165	7	11.7	0.60	0.17
	CEN3-MAT	273	138	4	7.6	0.53	0.19
	MAT-RAD18	214	201	3	19.3	0.16	0.0002

NPDs expected (NPDexp) are the numbers of four-strand double crossovers expected in the absence of interference (Papazian, 1952; Chua and Roeder, 1997). The NPD ratio is the number of NPDs observed divided by the number of NPDs expected. To calculate the proportion of NPDexp, the Papazian equation, $NPD = 1/2[1 - TT - (1 - 3TT/2)^{2/3}]$ (Papazian, 1952), was used, where NPD is the proportion of NPDexp, and TT is the proportion of TTs observed. The p value is the likelihood that the difference between the number of NPDs observed and the number expected in the absence of interference is attributable to chance (Chua and Roeder, 1997). PD, parental ditype; TT, tetratype; NPD, nonparental ditype.

by a helicase. Since neither Hop2 nor Mnd1 appear to have helicase motifs, they might function to recruit or aid a helicase. It is also possible that Hop2/Mnd1 are involved in dissociating nonhomologous sequences through a mechanism as yet unknown.

The requirement for an accessory factor in Dmc1/Rad51-mediated pairing and recombination is not surprising. Rad51-promoted strand exchange in vitro requires a host of proteins including RPA, Rad52, Rad54, Rad55, and Rad57 (Symington, 2002). However, the existence of a protein involved specifically in distinguishing homologous versus nonhomologous sequences was not anticipated.

Evidence for a Rad51-Only Meiotic Recombination Pathway

Overproduction of Rad51 suppresses the sporulation defect and restores spore viability in the *dmc1* mutant. It also increases the amount of crossing over to nearly wild-type levels.

By the following three criteria, recombination in *dmc1* strains overproducing Rad51 is mechanistically similar to that in wild-type. First, crossovers display interference. Second, crossover formation partially depends on the Msh4 protein. Third, axial associations are observed in a *zip1* background. Thus, Rad51 can substitute for the functions of Dmc1 during meiosis. We therefore propose

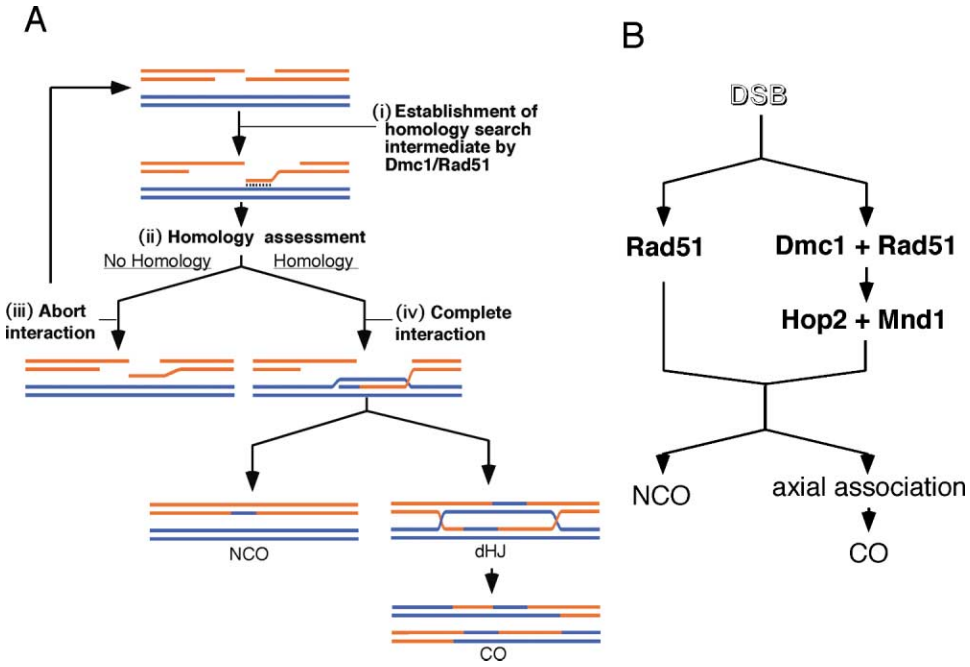

Figure 6. Cycle of Homology Searching and Meiotic Recombination Pathways

(A) The cycle of homology searching. We propose that the homology search process involves four important steps. (Ai) Two regions of DNA are physically aligned side by side; they could be on homologous or nonhomologous chromosomes. This step is most likely carried out by Rad51/Dmc1. The short vertical lines represent an unstable interaction between a DNA duplex and a single-stranded region. (Aii) The similarity of the two regions is assessed. (Aiii) If two aligned regions are not homologous, the interaction is aborted and another cycle starts from step (Ai) again. (Aiv) If two aligned regions are homologous, the recombination reaction proceeds, and recombinants are formed. NCO, noncrossover; CO, crossover; dHJ, double-Holliday junction.

(B) Meiotic recombination pathways. In this model, we propose that (1) Dmc1 and Hop2/Mnd1 act in the same pathway, with Dmc1 acting first; (2) Rad51 does not need Dmc1 and/or Hop2/Mnd1 to repair DSBs and form axial associations; (3) The Dmc1-dependent pathway likely requires Rad51 (see Discussion for details).

the existence of a Dmc1-independent, Rad51-dependent meiotic recombination pathway, which will hereafter be referred to as the "Rad51-only pathway" (Figure 6B).

It is reasonable to propose that Rad51 independently acts as a recombinase in the absence of Dmc1, since DSBs are repaired without Dmc1 during vegetative growth. Even meiotic DSBs can be repaired in the absence of Dmc1, at least in certain strain backgrounds (Figure 1). In the BR2495 background, the *dmc1* mutant sporulates, and ~20% of the spores formed are viable (Rockmill et al., 1995). In a *dmc1 spo13* double mutant, dyads (two-spored asci) are formed, and the spores are ~60% viable (Rockmill et al., 1995). Under these circumstances, crossing over is reduced more than 4-fold, suggesting that the spore inviability observed in diploids is the result of a reduction in crossing over and not due to a failure of DSB repair.

The phenotype of the *dmc1* mutant is different in different yeast strain backgrounds. The *dmc1* mutant is completely arrested at prophase I in the SK1 background (Bishop et al., 1992), whereas spores are formed and ~20% are viable in the BR2495 strain background (Rockmill et al., 1995). In fission yeast, the absence of Dmc1 does not decrease spore viability but greatly reduces crossing over (Fukushima et al., 2000). Furthermore, spore viability of the *dmc1* mutant is sensitive to the copy number of Rhp51, the fission yeast homolog of Rad51 (heterozygous disruption of *rhp51*$^+$ reduces spore viability in the *dmc1* mutant to 10%). Our finding that overproduction of Rad51 suppresses the *dmc1* defect raises the possibility that the differences in the *dmc1* mutant phenotype between different strain backgrounds or between different species are attributable simply to differences in the abundance of Rad51 protein during meiosis.

Unlike the Dmc1-dependent pathway, the Rad51-only pathway does not require the Hop2 or Mnd1 proteins (Figure 6B). Overproduction of Rad51 suppresses the sporulation defect and restores spore viability in both *hop2* and *mnd1* mutants. It also suppresses the *dmc1 hop2* double mutant to the same extent as it does in the *dmc1* mutant. Recent genome sequencing projects revealed that two model organisms (*Caenorhabditis elegans* and *Drosophila melanogaster*) have no Dmc1 homolog and are also missing Hop2 and Mnd1 homologs (http://www.wormbase.org/; Adams et al., 2000), suggesting that these organisms rely exclusively on the Rad51-only recombination pathway in meiosis.

What Does the Rad51-Only Pathway Tell Us About the Functions of Dmc1?

Whereas Rad51 appears to function in the absence of Dmc1, the converse is not true. Rad51 appears to be essential for the Dmc1-dependent recombination pathway. In the absence of Rad51, both tetrads and dyads (in the *rad51 spo13* double mutant) form, but the spores are not viable (Game, 1983) (our unpublished data). Dmc1 overproduction does not suppress the meiotic defect of *rad51*. Dmc1 localization to chromosomes appears to be largely dependent on Rad51 (Bishop, 1994). It is possible that Rad51 and Dmc1 work in a sequential manner in the Dmc1-dependent pathway, with Rad51 localizing to chromosomes first.

Previous studies suggest that Dmc1 specifically promotes recombination between homologous chromatids as opposed to recombination between sister chromatids (Schwacha and Kleckner, 1997). Here, we show that a *dmc1* mutant overproducing Rad51 undergoes approximately wild-type levels of crossing over. Since wild-type and *dmc1* overproducing Rad51 presumably form the same number of meiotic DSBs, recombination must take place preferentially between homologs in both cases. This leads to the idea that Dmc1 is not unique in specifically promoting interhomolog recombination. In fact, our data indicate that Dmc1 is also important in promoting recombination between sister chromatids.

Bishop and colleagues have recently proposed a model in which the coordinated action of Rad51 and Dmc1 is necessary for interference (Shinohara et al., 2003). In the present study, the *dmc1* mutant overproducing Rad51 shows moderate levels of crossover interference in two intervals and a wild-type level of interference in a third interval, indicating that an interference mechanism is still active. Consistent with this notion, the spore viability of the *dmc1* mutant overproducing Rad51 is partially dependent on Msh4, which is a protein necessary for the control of crossover distribution as well as for wild-type levels of crossing over (Novak et al., 2001). These data indicate that the involvement of Dmc1 in recombination is not essential for crossover interference. This conclusion is consistent with the observation that flies and worms, which rely on the Rad51-only recombination pathway, exhibit strong interference.

Dmc1, Hop2, and Mnd1 are all required for the formation of axial associations in the *zip1* mutant (Rockmill et al., 1995; Leu et al., 1998; our unpublished data). Here we show that overproducing Rad51 restores axial associations in the *dmc1 zip1* double mutant. Thus, the Rad51-only pathway as well as the Dmc1-dependent pathway work to establish a recombination intermediate that is represented cytologically by axial associations (Figure 6B).

What Is the Difference between the Rad51 and Dmc1 Proteins?

Importantly, it takes an abundance of Rad51 to substitute for Dmc1 function, suggesting that the difference is mostly in the level of recombination efficiency. Then why does meiotic recombination happen poorly with Rad51 alone, unlike the situation during vegetative growth? One possibility is that meiosis-specific chromosome structure interferes with the action of Rad51. Two candidate proteins that may create an obstacle to Rad51-mediated recombination are Red1 and Mek1, components of the lateral elements of the SC (Smith and Roeder, 1997; Bailis and Roeder, 1998). In the absence of Red1 or Mek1, DSBs can be repaired without Dmc1 (Xu et al., 1997), suggesting that some meiosis-specific aspect of chromosome structure imposes a requirement for Dmc1, Hop2, and Mnd1 (and possibly other proteins) for efficient meiotic recombination.

Experimental Procedures

Yeast Strains and Plasmids

All yeast strains used are either haploids or diploids whose parents are isogenic with the haploid strain BR1919-8B (Rockmill and

Roeder, 1990). The wild-type diploid, called S3246, is heterozygous at *MAT* and homozygous for *his4-260, leu2-3, 112 ura3-1 trp1-289 ade2-1*, and *thr1-4* (Tsubouchi and Roeder, 2002). Isogenic derivatives of S3246 were constructed by mating appropriate haploids; isogenic haploids were generated by transformation and/or by genetic crosses. To construct a haploid strain capable of sporulation, *MAT***a** was integrated at the *THR1* locus in a haploid *MAT*α version of BR1919-8B (Rockmill and Roeder, 1998). BR1919-8B strains carrying *lys2* and *HIS4* were obtained from B. Rockmill and J. Novak (Yale University, New Haven, CT), respectively.

To make a strain for measuring crossing over on chromosome III, *ADE2* was inserted upstream of the promoter region of *RAD18*, and *TRP1* was inserted at *CEN3* using plasmids described previously (Chua and Roeder, 1997). A diploid was made by mating the following haploids: *MAT***a** *HIS4 CEN3::TRP1 RAD18::ADE2 leu2-3,112 ura3-1 ade2-1 thr1-4 trp1-289 LYS2 dmc1::KAN* and *MAT*α *his4-260 leu2-3,112 ura3-1 ade2-1 thr1-4 trp1-289 lys2 dmc1::KAN*. Diploid strains carrying YCp-*DMC1* or YEpFAT4-*RAD51* were used for crossover analysis.

To create YEpFAT4-*RAD51,* a 3.7 kb BamHI fragment carrying *RAD51* from YEp51 (Shinohara et al., 1992) was inserted at the BamHI site of YEpFAT4 (Conrad et al., 1990). To create YEpFAT4-*DMC1*, a 3.2 kb XbaI fragment carrying *DMC1* from pNKY1173 (Bishop et al., 1992) was inserted at the XbaI site of YEpFAT4. A 3.7 kb BamHI fragment carrying *RAD51* from YEp51 was inserted at the BamHI site of YCp50 (Johnston and Davis, 1984) to make YCp-*RAD51*. A 3.2 kb XbaI fragment carrying *DMC1* from pNKY1173 was inserted at the XbaI site of YCplac111 (Gietz and Sugino, 1988) to make YCp-*DMC1*. YCp-*HOP2* is a single copy plasmid carrying the *HOP2* gene, formerly called pL13 (Leu et al., 1998). Plasmids introducing the following gene disruptions were described previously: *hop2::ADE2* (Tsubouchi and Roeder, 2002), *mnd1::KAN* (Tsubouchi and Roeder, 2002), *ndt80::LEU2* (Tung et al., 2000), *rad51::URA3* (Rockmill et al., 1995), *rad51::hisG* (Shinohara et al., 1992), *dmc1::URA3* (Bishop et al., 1992), *msh4::ADE2* (Ross-Macdonald and Roeder, 1994), and *zip1::LYS2* (Sym and Roeder, 1994). *dmc1::KAN* was engineered by PCR-mediated gene disruption; the *kanMX* cassette was used to replace the entire *DMC1* open reading frame including the start and stop codons (Longtine et al., 1998).

Cytology

Meiotic chromosomes were surface spread, and immunostaining and FISH were carried out as described previously (Sym et al., 1993; Chua and Roeder, 1998). Rabbit anti-Red1 antibody was used at a 1:500 dilution (Smith and Roeder, 1997). Rabbit anti-Zip1 antibody was used at a 1:100 dilution (Sym et al., 1993).

Homolog pairing and polycomplex formation were measured in an *NDT80* background as well as in *ndt80* strains. To measure homolog pairing in *NDT80* cells at prophase I, the Red1 protein was immunostained, and chromosome III was localized by FISH in the same spreads (Leu et al., 1998). Levels of homolog pairing similar to those shown in Figure 2B were observed in *hop2*, *hop2 dmc1*, and *hop2 rad51* mutants carrying a wild-type *NDT80* gene (data not shown). Polycomplex formation was measured in wild-type, *hop2*, *rad51*, *dmc1*, *hop2 rad51*, and *hop2 dmc1* strains carrying a wild-type *NDT80* gene. Levels of polycomplex formation similar to those shown in Figure 2D were observed (data not shown).

Measuring Sporulation

Patches were made from a single colonies of strains on YPD plates (or on selection plates when necessary) and incubated at 30°C over night. The plates were replica plated onto sporulation medium and incubated at 30°C for 2 or 3 days. For each strain, spore formation was measured in three independent experiments, with at least 300 cells scored in each experiment.

Acknowledgments

We thank Douglas Bishop, Janet Novak, and Beth Rockmill for providing plasmids and yeast strains. We are grateful to Jennifer Fung, Gillian Hooker, Neal Mitra, Beth Rockmill, and Tomomi Tsubouchi for comments on the manuscript. H.T. especially thanks Beth Rockmill and Tomomi Tsubouchi for critical reading of an early version of the manuscript. This work was supported by the Howard Hughes Medical Institute.

Received: July 30, 2003
Revised: October 10, 2003
Accepted: October 17, 2003
Published: December 8, 2003

References

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., Scherer, S.E., Li, P.W., Hoskins, R.A., Galle, R.F., et al. (2000). The genome sequence of *Drosophila melanogaster*. Science *287*, 2185–2195.

Agarwal, S., and Roeder, G.S. (2000). Zip3 provides a link between recombination enzymes and synaptonemal complex proteins. Cell *102*, 245–255.

Allers, T., and Lichten, M. (2001). Differential timing and control of noncrossover and crossover recombination during meiosis. Cell *106*, 47–57.

Bailis, J.M., and Roeder, G.S. (1998). Synaptonemal complex morphogenesis and sister-chromatid cohesion require Mek1-dependent phosphorylation of a meiotic chromosomal protein. Genes Dev. *12*, 3551–3563.

Bailis, J.M., and Roeder, G.S. (2000). The pachytene checkpoint. Trends Genet. *16*, 395–403.

Baumann, P., Benson, F.E., and West, S.C. (1996). Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. Cell *87*, 757–766.

Bishop, D.K. (1994). RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell *79*, 1081–1092.

Bishop, D.K., Park, D., Xu, L., and Kleckner, N. (1992). *DMC1*: a meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell *69*, 439–456.

Chu, S., and Herskowitz, I. (1998). Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. Mol. Cell *1*, 685–696.

Chua, P.R., and Roeder, G.S. (1997). Tam1, a telomere-associated meiotic protein, functions in chromosome synapsis and crossover interference. Genes Dev. *11*, 1786–1800.

Chua, P.R., and Roeder, G.S. (1998). Zip2, a meiosis-specific protein required for the initiation of chromosome synapsis. Cell *93*, 349–359.

Conrad, M.N., Wright, J.H., Wolf, A.J., and Zakian, V.A. (1990). Rap1 protein interacts with yeast telomeres in vivo: overproduction alters telomere structure and decreases chromosome stability. Cell *63*, 739–750.

de Massy, B., Baudat, F., and Nicolas, A. (1994). Initiation of recombination in *Saccharomyces cerevisiae* haploid meiosis. Proc. Natl. Acad. Sci. USA *91*, 11929–11933.

Dresser, M., Ewing, D., Conrad, M., Dominguez, A., Barstead, R., Jiang, H., and Kodadek, T. (1997). *DMC1* functions in a meiotic pathway that is largely independent of the *RAD51* pathway. Genetics *147*, 533–544.

Fukushima, K., Tanaka, Y., Nabeshima, K., Yoneki, T., Tougan, T., Tanaka, S., and Nojima, H. (2000). Dmc1 of *Schizosaccharomyces pombe* plays a role in meiotic recombination. Nucleic Acids Res. *28*, 2709–2716.

Game, J.C. (1983). Radiation-sensitive mutants and repair in yeast. In Yeast Genetics, J.F.T. Spencer, D.M. Spencer, and A.R.W. Smith, eds. (New York: Springer-Verlag), pp. 109–137.

Gerton, J.L., and DeRisi, J.L. (2002). Mnd1p: an evolutionarily conserved protein required for meiotic recombination. Proc. Natl. Acad. Sci. USA *99*, 6895–6900.

Gietz, R.D., and Sugino, A. (1988). New yeast - *Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene *74*, 527–534.

Gilbertson, L.A., and Stahl, F.W. (1994). Initiation of meiotic recombination is independent of interhomologue interactions. Proc. Natl. Acad. Sci. USA *91*, 11934–11937.

Haber, J.E., Leung, W.-Y., Borts, R.H., and Lichten, M. (1991). The frequency of meiotic recombination in yeast is independent of the number and position of homologous donor sequences: implications for chromosome pairing. Proc. Natl. Acad. Sci. USA *88*, 1120–1124.

Hong, E.L., Shinohara, A., and Bishop, D.K. (2001). *Saccharomyces cerevisiae* Dmc1 protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous supercoiled duplex DNA. J. Biol. Chem. *276*, 41906–41912.

Hunter, N., and Kleckner, N. (2001). The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. Cell *106*, 59–70.

Jinks-Robertson, S., and Petes, T.D. (1985). High-frequency meiotic gene conversion between repeated genes on nonhomologous chromosomes in yeast. Proc. Natl. Acad. Sci. USA *82*, 3350–3354.

Johnston, H.M., and Davis, R.W. (1984). Sequences that regulate the divergent *GAL1–GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *4*, 1440–1448.

Keeney, S. (2001). Mechanism and control of meiotic recombination initiation. Curr. Biol. *52*, 1–53.

Leu, J.-Y., Chua, P.R., and Roeder, G.S. (1998). The meiosis-specific Hop2 protein of *S. cerevisiae* ensures synapsis between homologous chromosomes. Cell *94*, 375–386.

Lichten, M., Borts, R.H., and Haber, J.E. (1987). Meiotic gene conversion and crossing over between dispersed homologous sequences occurs frequently in *Saccharomyces cerevisiae*. Genetics *115*, 233–246.

Loidl, J., Klein, F., and Scherthan, H. (1994). Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. J. Cell Biol. *125*, 1191–1200.

Longtine, M.S., McKenzie, A., III, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Phillippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast *14*, 953–961.

Nag, D.K., Scherthan, H., Rockmill, B., Bhargava, J., and Roeder, G.S. (1995). Heteroduplex DNA formation and homolog pairing in yeast meiotic mutants. Genetics *141*, 75–86.

Novak, J.E., Ross-Macdonald, P., and Roeder, G.S. (2001). The budding yeast Msh4 protein functions in chromosome synapsis and the regulation of crossover distribution. Genetics *158*, 1013–1025.

Ogawa, T., Yu, X., Shinohara, A., and Egelman, E.H. (1993). Similarity of the yeast Rad51 filament to the bacterial RecA filament. Science *259*, 1896–1899.

Papazian, H.P. (1952). The analysis of tetrad data. Genetics *37*, 175–188.

Peoples, T.L., Dean, E., Gonzalez, O., Lambourne, L., and Burgess, S.M. (2002). Close, stable homolog juxtaposition during meiosis in budding yeast is dependent on meiotic recombination, occurs independently of synapsis, and is distinct from DSB-independent pairing contacts. Genes Dev. *16*, 1682–1695.

Rabitsch, K.P., Toth, A., Galova, M., Schleiffer, A., Schaffner, G., Aigner, E., Rupp, C., Penkner, A.M., Moreno-Borchart, A.C., Primig, M., et al. (2001). A screen for genes required for meiosis and spore formation based on whole-genome expression. Curr. Biol. *11*, 1001–1009.

Rockmill, B., and Roeder, G.S. (1990). Meiosis in asynaptic yeast. Genetics *126*, 563–574.

Rockmill, B., and Roeder, G.S. (1998). Telomere-mediated chromosome pairing during meiosis in budding yeast. Genes Dev. *12*, 2574–2586.

Rockmill, B., Sym, M., Scherthan, H., and Roeder, G.S. (1995). Roles for two RecA homologs in promoting meiotic chromosome synapsis. Genes Dev. *9*, 2684–2695.

Roeder, G.S. (1997). Meiotic chromosomes: it takes two to tango. Genes Dev. *11*, 2600–2621.

Ross-Macdonald, P., and Roeder, G.S. (1994). Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell *79*, 1069–1080.

Schwacha, A., and Kleckner, N. (1994). Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell *76*, 51–63.

Schwacha, A., and Kleckner, N. (1997). Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell *90*, 1123–1136.

Shinohara, A., Ogawa, H., and Ogawa, T. (1992). Rad51 protein involved in repair and recombination in *S. cerevisiae* is a *RecA*-like protein. Cell *69*, 457–470.

Shinohara, A., Gasior, S., Ogawa, T., Kleckner, N., and Bishop, D.K. (1997). *Saccharomyces cerevisiae recA* homologues *RAD51* and *DMC1* have both distinct and overlapping roles in meiotic recombination. Genes Cells *2*, 615–629.

Shinohara, M., Gasior, S.L., Bishop, D.K., and Shinohara, A. (2000). Tid1/Rdh54 promotes colocalization of Rad51 and Dmc1 during meiotic recombination. Proc. Natl. Acad. Sci. USA *97*, 10814–10819.

Shinohara, M., Sakai, K., Shinohara, A., and Bishop, D.K. (2003). Crossover interference in *Saccharomyces cerevisiae* requires a *TID1/RDH54*- and *DMC1*-dependent pathway. Genetics *163*, 1273–1286.

Smith, A.V., and Roeder, G.S. (1997). The yeast Red1 protein localizes to the cores of meiotic chromosomes. J. Cell Biol. *136*, 957–967.

Sun, H., Treco, D., and Szostak, J.W. (1991). Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell *64*, 1155–1161.

Sung, P. (1994). Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast Rad51 protein. Science *265*, 1241–1243.

Sym, M., and Roeder, G.S. (1994). Crossover interference is abolished in the absence of a synaptonemal complex protein. Cell *79*, 283–292.

Sym, M., Engebrecht, J., and Roeder, G.S. (1993). Zip1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell *72*, 365–378.

Symington, L.S. (2002). Role of *RAD52* epistasis group genes in homologous recombination and double-strand break repair. Microbiol. Mol. Biol. Rev. *66*, 630–670.

Trelles-Sticken, E., Loidl, J., and Scherthan, H. (1998). Bouquet formation in budding yeast: initiation of recombination is not required for meiotic telomere clustering. J. Cell Sci. *112*, 651–658.

Trelles-Sticken, E., Conrad, M.N., Dresser, M.E., and Scherthan, H. (2000). Meiotic telomere protein Ndj1p is required for meiosis-specific telomere distribution, bouquet formation and efficient homologue pairing. J. Cell Biol. *151*, 95–106.

Tsang, S.S., Chow, S.A., and Radding, C.M. (1985). Networks of DNA and RecA protein are intermediates in homologous pairing. Biochemistry *24*, 3226–3232.

Tsubouchi, H., and Roeder, G.S. (2002). The Mnd1 protein forms a complex with Hop2 to promote homologous chromosome pairing and meiotic double-strand break repair. Mol. Cell. Biol. *22*, 3078–3088.

Tung, K.-S., Hong, E., and Roeder, G.S. (2000). The pachytene checkpoint prevents accumulation and phosphorylation of the meiosis-specific transcription factor Ndt80. Proc. Natl. Acad. Sci. USA *97*, 12187–12192.

Weiner, B.M., and Kleckner, N. (1994). Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. Cell *77*, 977–991.

Xu, L., Weiner, B.M., and Kleckner, N. (1997). Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev. *11*, 106–118.

Zickler, D., and Kleckner, N. (1999). Meiotic chromosomes: integrating structure and function. Annu. Rev. Genet. *33*, 603–754.