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The Meiosis-Specific Hop2 Protein of *S. cerevisiae* Ensures Synapsis between Homologous Chromosomes

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Summary

The *hop2* mutant of *S. cerevisiae* displays a novel phenotype: meiotic chromosomes form nearly wild-type amounts of synaptonemal complex, but most chromosomes are engaged in synapsis with nonhomologous partners. The meiosis-specific Hop2 protein localizes to chromosomes prior to and during synapsis and in the absence of the double-strand breaks that initiate recombination. *hop2* strains sustain a wild-type level of meiotic double-strand breaks, but these breaks remain unrepaired. The *hop2* mutant arrests at the pachytene stage of meiotic prophase with the RecA-like protein Dmc1 located at numerous sites along synapsed chromosomes. We propose that the Hop2 protein functions to prevent synapsis between nonhomologous chromosomes.

Introduction

During meiotic prophase, homologous chromosomes pair with each other, undergo genetic recombination, and engage in synaptonemal complex (SC) formation. These interhomolog interactions are necessary to establish chiasmata, which are chromatin bridges between homologs that correspond in position to the sites of crossovers. Chiasmata hold homologs together at metaphase of meiosis I and therefore ensure the correct segregation of chromosomes at anaphase I. If homologs fail to interact (reviewed by Roeder, 1997), or if crossing over takes place between nonhomologous chromosomes (Parker, 1969; Jinks-Robertson et al., 1997), homologs undergo nondisjunction at meiosis I, and inviable meiotic products are generated.

A eukaryotic chromosome must find its homolog within a meiotic nucleus that contains a large and complex mixture of DNA sequences. The problem of homolog pairing is exacerbated by the fact that the eukaryotic genome contains dispersed repeated DNA sequences, such as transposable elements. These repeats offer the potential for recombination and synapsis between homologous sequences on otherwise nonhomologous chromosomes. In principle, at least four different mechanisms might operate to prevent these ectopic interactions and confine interactions to homologs. First, there may be competition between allelic and ectopic interactions, with homologs usually winning this competition due to the greater extent of shared homology. Second, mechanisms might operate to prevent interactions at ectopic sites; for example, repeated DNA sequences could be packaged into a chromatin structure that renders them inaccessible to recombination enzymes. Third, it might be possible to eliminate or reverse ectopic interactions as meiosis progresses. A fourth possibility is that chromosomes are nonrandomly positioned within the nucleus such that homologs are predisposed to interact with each other.

In *Saccharomyces cerevisiae*, fluorescent in situ hybridization (FISH) using chromosome-specific probes has demonstrated that the pairing (i.e., roughly parallel alignment) of homologous chromosomes precedes and is largely independent of genetic recombination and SC formation (reviewed by Roeder, 1997). These studies also indicate that sites capable of pairing are numerous and widely dispersed along the lengths of chromosomes (Weiner and Kleckner, 1994). The observed high frequencies of gene conversion between dispersed repeats suggest that meiotic pairing involves a genome-wide homology search in which each DNA sequence is able to probe many other sequences in the yeast genome (e.g., Goldman and Lichten, 1996). It has been suggested that pairing involves the formation of unstable side-by-side paranemic joints between intact DNA duplexes (Weiner and Kleckner, 1994; Kleckner, 1996). The RecA strand exchange protein of *Escherichia coli* is capable of forming such paranemic joints (West, 1992). However, in yeast, a significant level of meiotic homolog pairing occurs in the absence of all four RecA homologs (Kleckner, 1996). Thus, the molecular mechanisms responsible for pairing remain mysterious.

Chromosome pairing culminates in the formation of the SC, a ribbon-like structure that holds homologs in close apposition along their lengths during the pachytene stage of meiotic prophase (reviewed by Roeder, 1997). Each SC consists of two lateral elements, corresponding to the proteinaceous backbones of the individual chromosomes within the complex. Lateral elements are referred to as axial elements prior to their incorporation into SC. When synapsis (i.e., SC formation) is complete, the two lateral elements are separated by a uniform distance and held together by proteins that constitute the central region of the SC. In budding yeast, the Zip1 protein has been shown to be a component of the central region (Sym et al., 1993; Sym and Roeder, 1995; Tung and Roeder, 1998). In the absence of Zip1, full-length axial elements are assembled and paired, but not synapsed (Sym et al., 1993). Each pair of unsynapsed axial elements is connected at a few sites, called axial associations, that are believed to be sites where synapsis normally initiates (Rockmill et al., 1995; Chua and Roeder, 1998). Recently, the Zip2 protein has been shown to localize to axial associations and to be required for the initiation of synapsis (Chua and Roeder, 1998). Several observations suggest that axial associations correspond to the sites of meiotic recombination events (Rockmill et al., 1995; Chua and Roeder, 1998).

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In *S. cerevisiae*, meiotic recombination initiates with double-strand breaks (DSBs) (reviewed by Roeder, 1995, 1997). The ends of the breaks are processed to expose single-stranded tails with 3′ termini. These tails invade homologous sequences on a nonsister chromatid to form a joint molecule, whose resolution results in gene conversion and/or crossing over. Many of the gene products required for DSB formation and processing in yeast have been identified (reviewed by Roeder, 1995, 1997). Nine different gene products have been shown to be required for the formation of DSBs. At least three proteins, Sae2/Com1, Rad50, and Mre11, are required to process DSBs to expose single-stranded tails. The Rad51, Dmc1, Rad55, and Rad57 proteins are required for strand invasion, and consequently, for the formation of joint molecules.

Here, we report the characterization of mutants in a novel yeast gene, called *HOP2* (for *h*omologous *p*airing). The phenotype of the *hop2* null mutant is distinct from that of other yeast meiotic mutants previously characterized: chromosomes undergo nearly wild-type amounts of SC formation, but most synapsis involves nonhomologous chromosomes. The Hop2 protein localizes to chromosomes prior to and during synapsis and in the absence of genetic recombination. We propose that Hop2p serves to discriminate interactions between homologs from interactions between nonhomologous chromosomes.

Results

Identification and Cloning of *HOP2*

The *hop2-1* mutant was isolated in a screen for mutants defective in meiotic gene conversion (see Experimental Procedures). Diploids homozygous for *hop2-1* fail to sporulate. The wild-type *HOP2* gene was cloned from a yeast genomic library based on complementation of the sporulation defect. *HOP2* was localized to a region of 1.3 kbp by subcloning and analysis of overlapping *hop2*-complementing clones. Comparison of DNA sequence derived from the 1.3 kbp fragment with sequences from the GenBank database indicates that the *HOP2* gene corresponds to chromosomal locus YGL033w (Tettelin et al., 1997). Cloning and sequencing of *HOP2* cDNAs revealed that *HOP2* contains two exons and an intron with a nonconsensus 5′ splice site (5′-GTTAAGT-3′ instead of 5′-GTAPyGT-3′). The endpoints of the *HOP2* exons and intron have been confirmed by RNase protection experiments and by mutational analysis of the initiation codon, the 5′ splice site, and the branch point sequence (unpublished data). The *HOP2* gene is predicted to encode a protein of 203 amino acids in length with no significant similarity to other proteins in available databases (Figure 1A).

HOP2 Expression Is Meiosis-Specific

Expression of the *HOP2* gene was measured in a strain heterozygous for a *hop2::lacZ* translational fusion gene. β-galactosidase assays were performed during vegetative growth and at various times throughout meiosis. There was little or no expression in vegetative cells. Upon transfer to sporulation medium to induce meiosis,

A

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ATGGCACCGAAAAAGAAATCAAACGATAGAGCCATTCAGGCAAAAGGCTCAGAAGgttaa 60
M  A  P  K  K  K  S  N  D  R  A  I  Q  A  K  G  S  E  A
gtatttttttacatgctcatcaaataccgccattactaacaattgtaccccgggtatttt 120
atcagCTGAACAATTAATAGAAGATTACTTGGTGTCACAATATAAACCATTTTCTGTAAA 180
       E  Q  L  I  E  D  Y  L  V  S  Q  Y  K  P  F  S  V  N
TGATATAGTTCAGAATTTGCACAATAAAGTGACAAAAACAACAGCAACCAAAGCATTAGA 240
 D  I  V  Q  N  L  H  N  K  V  T  K  T  T  A  T  K  A  L  E
AAATCTCGTTAACGAGAAACGCATAGTGTCTAAAACCTTCGCAAAGATTATAATTTACTC 300
 N  L  V  N  E  K  R  I  V  S  K  T  F  A  K  I  I  I  Y  S
TTGTAATGAACAAGATACAGCATTACCAAGCAATATTGATCCTTCTCAGTTTGATTTTGA 360
 C  N  E  Q  D  T  A  L  P  S  N  I  D  P  S  Q  F  D  F  E
AACTGTATTGCAACTGAGAAATGATTTAATAGAACTAGAGAGAGATAAATCAACGGCCAA 420
 T  V  L  Q  L  R  N  D  L  I  E  L  E  R  D  K  S  T  A  K
AGACGCTCTTGACTCTGTAACTAAAGAACCTGAAAATGAAGATCTCCTAACAATCATTGA 480
 D  A  L  D  S  V  T  K  E  P  E  N  E  D  L  L  T  I  I  E
AAACGAAGAAAACGAGTTGAAAAAGATTGAATCAAAATTACAAAGTCTACAGGATGATTG 540
 N  E  E  N  E  L  K  K  I  E  S  K  L  Q  S  L  Q  D  D  W
GGATCCAGCAAATGATGAGATTGTCAAGCGGATAATGTCTGAAGATACGCTGCTACAAAA 600
 D  P  A  N  D  E  I  V  K  R  I  M  S  E  D  T  L  L  Q  K
AGAAATCACGAAGAGATCAAAGATTTGCAAAAAACCTAATTGCTACAATAAAGGACTCAG 660
 E  I  T  K  R  S  K  I  C  K  K  P  N  C  Y  N  K  G  L  S
TGTGCCCGAAAAATATGAATGA
 V  P  E  K  Y  E  *
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B

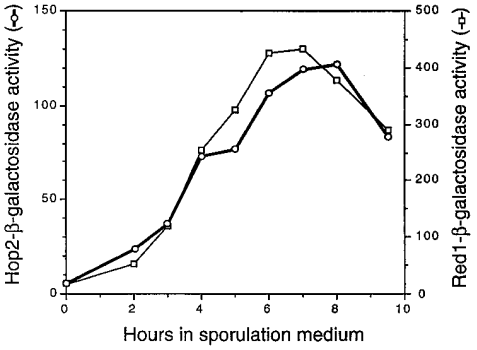


Figure 1. Predicted Hop2 Amino Acid Sequence and Analysis of Gene Expression

(A) The DNA sequence of the *HOP2* gene is presented with the nucleotides in the intron indicated in lower case. The predicted amino acid sequence of the Hop2 protein is shown below the DNA sequence.

(B) Production of Hop2 and Red1 fusion proteins was monitored in strains carrying *hop2::lacZ* (SYAB8) and *red1::lacZ* (SYAB9) translational fusion genes. β-galactosidase assays were performed on vegetative cells (0 hr of sporulation) and on aliquots of cells taken at regular intervals from sporulating cultures. Circles, Hop2p-β-galactosidase; squares, Red1p-β-galactosidase.

β-galactosidase activity increased rapidly and reached a maximum at 8 hr (Figure 1B). The timing of *HOP2* expression is similar to that of another meiosis-specific gene, *RED1*, which is expressed maximally at mid meiotic prophase (Thompson and Roeder, 1989). Thus, *HOP2* is a member of the early class of meiotic genes (Mitchell, 1994).

The *hop2* Null Mutant Arrests in Meiotic Prophase

When diploids homozygous for a *hop2* null mutation are introduced into sporulation medium, they fail to make tetrads. To determine the stage at which the *hop2* mutant is defective, cells at various time points in meiosis were stained with a DNA-binding dye [4′,6-diamidino-2-phenyl-indole (DAPI)] and viewed in the fluorescence microscope. Almost all cells in the mutant remain mononucleate, even at late time points when wild type has completed sporulation. For example, at 60 hr, 98.6% of *hop2* cells (YAB27) were mononucleate; at the same

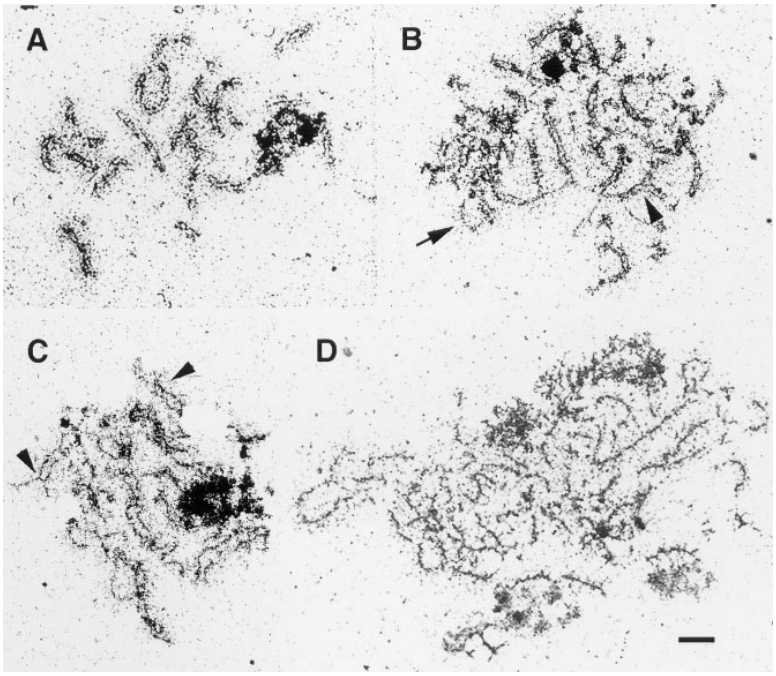


Figure 2. Electron Micrographs of Spread Meiotic Nuclei

Spread meiotic nuclei from (A) wild type (YAB36), (B) *hop2* (YAB27), (C) *zip1* (YAB89), and (D) *hop2 zip1* (YAB92). Spreads were prepared after 14 hr of sporulation. In (B), the arrowhead indicates a branched chromosome, and the arrow indicates an unsynapsed axial element. The arrowheads in (C) indicate axial associations. Scale bar = 1 μm.

time point in wild type (YAB36), 28.4% of cells were mononucleate, 8.4% were binucleate (had completed one division), and 63.2% tetranucleate (had completed both meiotic divisions). Data presented below demonstrate that *hop2* cells do enter meiosis; thus, the *hop2* mutant arrests in meiotic prophase prior to the first meiotic division.

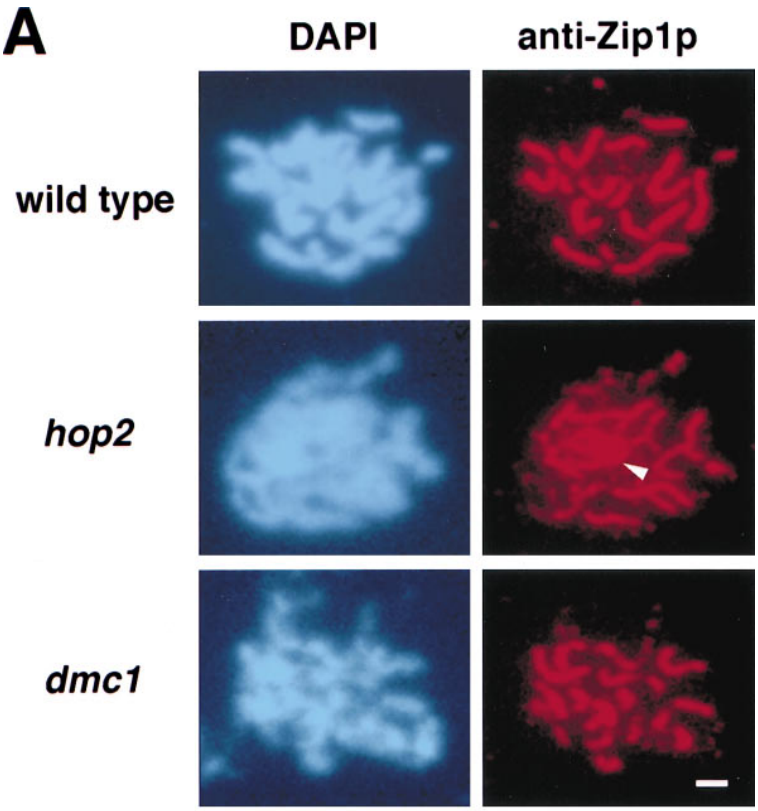
The *hop2* sporulation defect is bypassed by a *spo11* mutation, which prevents the formation of meiotic DSBs (Cao et al., 1990). After 72 hr in sporulation medium, 49% of *spo11 hop2* cells (YAB86) and 46% of *spo11* cells (YAB88) had formed asci, whereas no *hop2* cells (YAB27) had completed sporulation. This bypass by *spo11* suggests that meiotic arrest in *hop2* results from the operation of a meiotic checkpoint that is triggered by a defect in recombination and/or synapsis (Bishop et al., 1992; Sym et al., 1993; Lydall et al., 1996). Unlike the *dmc1* and *zip1* mutants that display strain-specific sporulation defects (Bishop et al., 1992; Sym and Roeder, 1994; Rockmill et al., 1995), the *hop2* mutant displays a uniform meiotic arrest in all strains tested, including SK1 and BR2495. Thus, meiotic arrest in *hop2* strains appears to be triggered by a different signal and/or effected by a different mechanism than the arrests observed in *zip1* and *dmc1* strains.

Chromosomes Synapse in the *hop2* Mutant

To examine the effect of *hop2* on SC formation, meiotic nuclei were surface spread, stained with silver nitrate, and examined in the electron microscope. In addition, surface-spread chromosomes were stained with antibodies to Zip1p and viewed in the fluorescence microscope. The *hop2* mutant assembles nearly wild-type amounts of SC (Figures 2A and 2B; Figure 3), but the nuclei in the mutant differ from those observed in wild type (Figure 2A) in two ways. First, unsynapsed axial elements and partially synapsed chromosomes are rarely seen in wild type, but they are frequently observed in the mutant (e.g., Figure 2B), especially at early times. Second, chromosomes in the mutant are difficult to separate from each other during spreading as if all chromosomes are connected (Figure 2B; Figure 3). This feature of *hop2* spreads is particularly pronounced at late times when chromosomes often appear to be branched (Figure 2B); such branches suggest that different parts of a single chromosome are synapsed with different partners.

To examine the kinetics of synapsis, spread chromosomes from different time points were stained with antibodies to Zip1p (Figure 3B). In wild type, the average intensity of anti-Zip1p staining per nucleus reaches a peak after about 12 hr in sporulation medium. At this time, the majority of wild-type cells are at the pachytene stage with full-length SCs. At 12 hr and earlier, the level of anti-Zip1p staining in the mutant is significantly lower than in wild type, indicating that synapsis is delayed in the absence of Hop2p. By 13 hr, the average intensity of fluoresence in the mutant is equivalent to that of wild type. At later times, the average intensity of Zip1p staining in *hop2* nuclei is higher than that of wild type because *hop2* cells arrest at pachytene and Zip1p staining persists, whereas wild type progresses through meiosis. At 24 and 44 hr of sporulation, when almost all wild-type cells have completed meiosis, most *hop2* nuclei still contain SCs.

Staining with anti-Zip1p antibodies also revealed that many *hop2* nuclei contain polycomplexes (Figure 3A, Figure 4), which are assemblies of SC proteins unassociated with chromatin. Polycomplexes have been observed in mutants defective in chromosome synapsis (Alani et al., 1990; Loidl et al., 1994; Prinz et al., 1997) and in strains overproducing Zip1p (Sym and Roeder, 1995). In *hop2* cells, polycomplexes coexist with SCs in a large fraction of cells (48% of 200 nuclei scored at 16 hr). The formation of polycomplexes in the *hop2* mutant



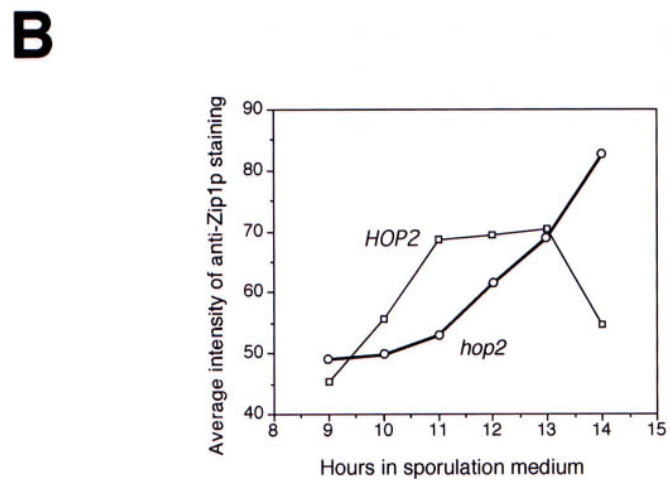


Figure 3. Zip1p Localization and Kinetics of Chromosome Synapsis

(A) Surface-spread pachytene nuclei from wild-type (YAB36), *hop2* (YAB27), and *dmc1* (YAB125) strains stained with DAPI (blue) and antibodies to Zip1p (red). The arrowhead indicates a polycomplex.

(B) Time course of synapsis in wild type and the *hop2* mutant. The average intensity of anti-Zip1p antibody staining per nucleus was determined as described in Experimental Procedures. At least 150 nuclei were scored at each time point for each strain. Squares, wild type (YAB36); circles, *hop2* (YAB27). The strains used to examine the kinetics of synapsis sporulate more slowly than those used in measurements of gene expression (Figure 1) and physical assays of recombination (Figure 5).

cannot be attributed entirely to the overproduction of Zip1p that occurs in *hop2* cells arrested at pachytene (data not shown) because polycomplexes are frequently observed at early times prior to extensive SC formation. These results are consistent with the observed delay in synapsis and aberrations in SC assembly.

Chromosomes Are Synapsed with Nonhomologous Partners in the *hop2* Mutant

The difficulty in separating chomosomes during spreading and the appearance of branched chromosomes raised the possibility that some or all SC formation in the *hop2* mutant involves nonhomologous chromosomes. To investigate this possibility, homolog pairing was assessed by FISH; for comparison, the *dmc1* mutant, which also displays a delay in synapsis and occasional unsynapsed axial elements (Rockmill et al., 1995), was also analyzed. Hybridization of a chromosome-specific probe to a spread nucleus produces a single hybridization signal (or two closely spaced signals) if homologs are paired, but two separate signals if homologs are unpaired. Homologs were paired in nearly 100% of pachytene nuclei from wild type (Table 1). Pairing in the *hop2* mutant ranged from 15% to 45%, depending on the chromosome tested (Table 1); pairing values in the *dmc1* strain were much higher, ranging from 53% to 73%. The substantial reduction in homolog pairing in the *hop2* mutant is consistent with the hypothesis that many of the SCs

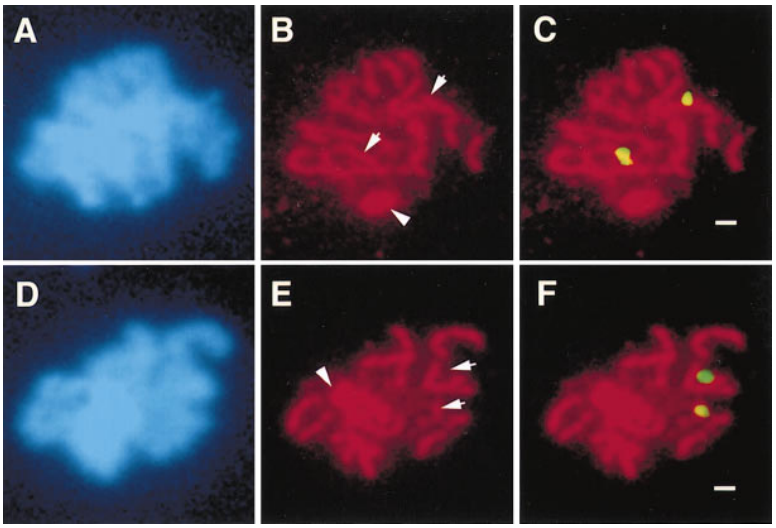


Figure 4. Unpaired FISH Dots Localize to Synapsed Chromosomes in the *hop2* Mutant

Spread nuclei from a *hop2* strain (YAB27) were stained with anti-Zip1p antibodies followed by hybridization with a chromosome III probe. Shown are two pachytene nuclei visualized by DAPI staining (A and D), staining with anti-Zip1p antibodies (B and E), and anti-Zip1p antibodies plus a chromosome III probe (C and F). The chromosomes III are unpaired in both nuclei. The arrows in (B) and (E) point to the sites of the FISH signals shown in (C) and (F). In (F), only one of the FISH dots (the lower one) colocalizes with SC; in (C), both FISH dots colocalize with SCs. The arrowheads indicate polycomplexes. Scale bar = 1 μm.

observed involve synapsis of nonhomologous chromosomes.

To confirm the occurrence of nonhomologous synapsis in the *hop2* mutant, spread meiotic nuclei were simultaneously labeled with anti-Zip1p antibodies and hybridized with a probe for chromosome III (Figure 4). In the *hop2* mutant, the two copies of chromosome III were unpaired in about 75% of the pachytene nuclei. Approximately 25% of unpaired FISH signals did not overlap with Zip1p staining, suggesting that these chromosomes were unsynapsed. The remaining 75% of unpaired FISH signals displayed significant (>50%) overlap with regions of Zip1 staining. In the *dmc1* mutant, chromosomes III were unpaired in 49% of pachytene nuclei; and only 19% of the unpaired FISH signals overlapped with Zip1p staining; some of this overlap is likely to be fortuitous. Overall, these data indicate that ~56% (.75 × 75%) of chromosomes III are engaged in nonhomologous synapsis in the *hop2* mutant, compared to ~9% (.49 × 19%) in the *dmc1* strain. In the *hop2* mutant, more than half of the chromosomes III appear to be engaged in synapsis with a nonhomologous chromosome or folded back on themselves such that different parts of the same chromosome are synapsed with each other.

Table 1. Homologous Pairing in Wild Type, *hop2*, and *dmc1*

	HOP2	*hop2*	*dmc1*
Chromosome III	100%	26%	53%
Chromosome V	97%	15%	53%
Chromosome VII	100%	45%	73%

The percentage of homologous pairing was assayed by FISH in wild type (YAB36) and in the *hop2* (YAB27) and *dmc1* (YAB125) mutants. At least 130 pachytene nuclei from each strain were scored in each experiment. FISH was carried out after 13 (for wild type) or 18 hr (for mutants) in sporulation medium, when most nuclei were in pachytene. Pairing did not increase at later time points in the *hop2* mutant (data not shown). Homolog pairing was measured only in nuclei at the pachytene stage as determined by DAPI staining (chromosome pairs that are condensed and synapsed appear as "sausages").

The *hop2* Mutant Fails to Repair DSBs

To examine the effect of *hop2* on meiotic recombination, physical assays were used to monitor recombination intermediates and products. Genomic DNA was collected from wild type and mutant at different time points after the induction of meiosis and assayed for exchange in an interval that includes the *ARG4* recombination hotspot (Goyon and Lichten, 1993). At early times, DSBs are evident in both wild type and mutant (Figure 5A). At later times in wild type, the DSB fragments decline in intensity, and the bands representing mature crossover products appear (Figure 5A). In the *hop2* mutant, however, the fragments indicative of DSBs persist much longer (though they are eventually degraded); mature crossover products are not detected, even at late time points (Figure 5A). Similar results were obtained at the *HIS4-LEU2* recombination hotspot (data not shown). These data demonstrate that recombination is not completed in *hop2* cells arrested in meiosis.

To examine the effect of *hop2* on the initiation of recombination, the level of meiotically induced DSBs was assessed in a *rad50S* strain background, in which DSBs accumulate (Alani et al., 1990). In the *hop2* mutant, DSB formation at the *HIS4-LEU2* hotspot (Cao et al., 1990) is similar to wild type with respect to the level of breakage and the kinetics of appearance of DSB products (Figure 5B). At 6–8 hr after the introduction into sporulation medium, DSB fragments represent an average of 19.6% of total DNA in wild type, compared to 15.4% in the mutant. In the same strains used to measure DSBs, the mutant displays an 11-fold reduction in commitment to gene conversion at *HIS4* (Table 2). The *hop2* mutant also sustains an approximately wild-type level of DSBs at the *ARG4* recombination hotspot (data not shown). Thus, the *hop2* defect in gene conversion cannot be attributed to a failure to initiate recombination.

The effect of *hop2* on the processing of DSBs to expose single-stranded tails was examined at the *HIS4-LEU2* hotspot. Genomic DNA from *hop2 RAD50* and *hop2 rad50S* cells was digested with restriction enzymes chosen to generate relatively small fragments such that changes in size can be readily detected by

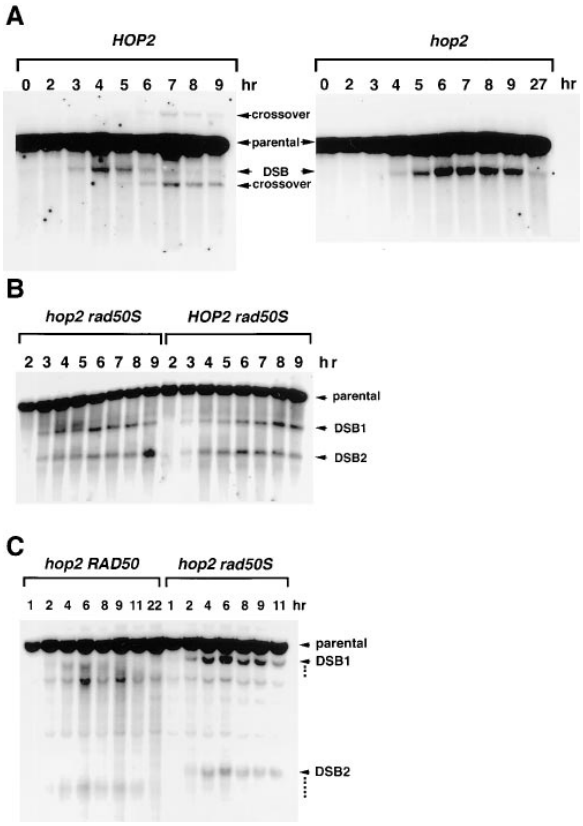


Figure 5. Physical Assays of Recombination Intermediates and Products

Genomic DNA was isolated from cells at the indicated times after transfer to sporulation medium.

(A) Time course of recombination at the *ARG4* locus in wild-type (SYAB1) and *hop2* (SYAB2) strains. Genomic DNA was digested with BamHI and BglII and probed with the 0.5 kb SacI fragment of *ARG4* (Goyon and Lichten, 1993). No crossover bands were detected in *hop2* even after a longer exposure (data not shown). The slight delay in the appearance of DSBs in the *hop2* mutant was not observed in other experiments.

(B) Accumulation of DSBs at the *HIS4-LEU2* hotspot in *rad50S* (SYAB4) and *hop2 rad50S* stains (SYAB5). DNA was digested with PstI and probed with the 0.4 kb XhoI-SalI fragment of *HIS4* (Cao et al., 1990).

(C) DSB processing in *hop2* (SYAB2) and *hop2 rad50S* (SYAB3) strains. DNA was digested with PvuI and NdeI and then probed with the 0.3 kb PvuI-XbaI fragment of *HIS4* (Bishop et al., 1992).

Southern blot analysis. Compared to DSB fragments from a *hop2 rad50S* strain, the DSB fragments in a *hop2 RAD50* strain are of lower molecular weight and more heterogeneous size (Figure 5C). At late time points, DSB products are still evident in the *hop2 rad50S* strain, but they can no longer be detected in the *hop2 RAD50* strain. These results indicate that DSBs are processed to expose single-stranded tails in the *hop2* mutant.

hop2 Has Variable Effects on the Commitment to Meiotic Gene Conversion

The effect of *hop2* on the commitment to meiotic gene conversion was measured in return-to-growth experiments in which sporulating cells were returned to growth medium after the induction of meiotic recombination.

Table 2. Effect of *hop2* on Commitment to Meiotic Gene Conversion

Yeast Strains	Locus	Frequency ($\times 10^{-4}$) *HOP2*	Frequency ($\times 10^{-4}$) *hop2*	Fold Decrease
YAB36, YAB27	*his4-280* *his4-260*	140.6	80.5	1.7
YAB36, YAB27	*leu2-3,112* *leu2-27*	9.4	1.0	9.4
YAB36, YAB27	*thr1-4* *thr1-1*	1.9	0.2	9.5
YAB36, YAB27	*trp1-289* *trp1-1*	0.9	0.7	1.3
SYAB1, SYAB2	*his4-B* *his4-X*	458.8	42.3	10.8
SYAB6, SYAB7	*arg4-nsp* *arg4-bgl*	1133.3	257.3	4.4
YAB45, YAB46	*his4-712* *his4-Hpa*	78.5	75.0	1.0
YAB51, YAB49	*his4-712* *his4-260*	356.5	35.0	10.2

Strains YAB36 and YAB27 are isogenic. Strains SYAB1, SYAB2, SYAB6, and SYAB7 are isogenic. Strains YAB45, YAB46, YAB49, and YAB51 are isogenic.

Conversion was measured in strains carrying two different mutant alleles at a single locus, such that intragenic recombination produces prototrophs. Depending on the locus and pair of heteroalleles examined, the *hop2* mutation had little or no effect or decreased recombination as much as 11-fold (Table 2). Even at a single locus (e.g., *HIS4*), the effect of *hop2* varied considerably depending on the pair of heteroalleles examined. This differential effect likely reflects the fact that DSBs in the mutant are repaired after the return to vegetative growth, whereas most DSBs in wild type are repaired meiotically. Differences in gene conversion tract length between vegetative and meiotic cells affect the fraction of intragenic recombinants recovered as prototrophs (Petes et al., 1991).

Axial Associations Are Not Observed in the Absence of Hop2p

During meiosis in wild type, synapsis initiates at axial associations (Chua and Roeder, 1998). However, axial associations are not detected in haploids (Rockmill et al., 1995), which undergo extensive nonhomologous synapsis (Loidl et al., 1991). The nonhomologous synapsis observed in haploids may be mechanistically related to the synapsis that occurs in *hop2* strains. To determine whether *hop2* affects the formation of axial associations, spread meiotic chromosomes from a *hop2 zip1* double mutant were stained with silver nitrate and examined in the electron microscope. In contrast to the axial elements observed in a *zip1* single mutant (Figure 2C), the axial elements observed in the double mutant are not obviously paired or connected by axial associations (Figure 2D). These results suggest that the *hop2* mutation affects the formation or stability of axial associations. An alternative explanation is that *hop2* affects only the distribution of axial associations. In wild type, each pair of homologs is connected to each other (and only to each other) by one or more axial associations. In

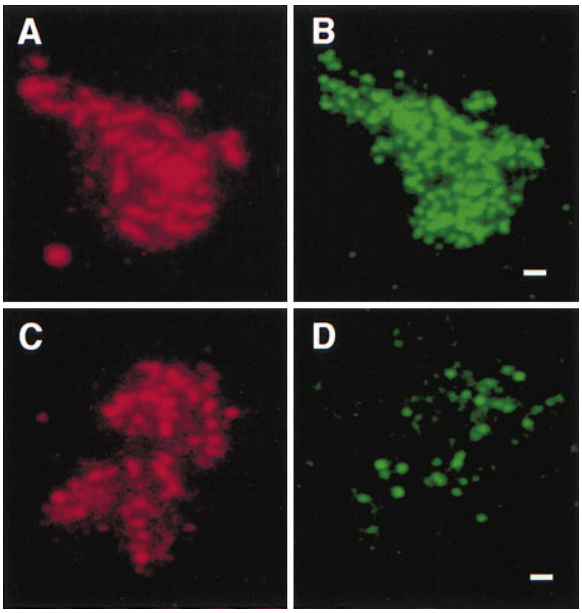


Figure 6. Localization of the Dmc1 Protein

Spread nuclei were stained with anti-Zip1p (red) and anti-Dmc1p antibodies (green). (A and B) Pachytene nucleus from the *hop2* mutant (YAB27). (C and D) Zygotene nucleus from wild type (YAB36). In wild type, the maximum number of Dmc1p foci is observed before chromosomes are fully synapsed. Scale bar = 1 μm.

the *hop2* mutant, however, each chromosome may be connected by axial associations to a number of different chromosomes. Multiple connections between heterologous chromosomes might exert tension on chromosomes during spreading, resulting in the disruption of axial associations.

Dmc1p Fails to Dissociate from Chromosomes in the *hop2* Mutant

Previous work has shown that the RecA-like proteins, Dmc1 and Rad51, localize to a number of discrete sites on meiotic chromosomes (Bishop, 1994). In our experiments, we observed an average of 27 (±12, 20 nuclei counted) Dmc1p-staining foci in wild-type cells after 10.5 hr in sporulation medium (Figure 6D), when ~56% of cells displayed Dmc1p-staining foci. Dmc1p does localize to chromosomes in the *hop2* mutant (Figure 6B), but the Dmc1p-staining pattern differs from that observed in wild type in three respects. First, Dmc1p-staining persists even after chromosomes have fully synapsed. Second, the Dmc1p foci observed in *hop2* cells are brighter than those in wild-type cells. Third, the average number of Dmc1p foci per nucleus is much higher than in wild type. We counted an average number of ~140 (±11, 30 nuclei examined) Dmc1 complexes, but this number is almost certainly an underestimate. Due to the large number of foci and the small area defined by a nuclear spread, two or more complexes in close proximity might appear as a single focus by immunofluorescence. Consistent with this notion, the size and intensity of Dmc1p-staining foci within a single nucleus are quite variable. Thus, in a *hop2* mutant, the number of Dmc1p-staining foci approaches the number of Dmc1p-dependent interhomolog recombination events (~80% of total [260] = ~210) as determined by genetic and physical analyses (Bishop et al., 1992; Rockmill et al., 1995). The increased number, intensity, and life span of Dmc1p-containing complexes in the *hop2* mutant are consistent with the results of physical assays indicating that recombination is blocked.

Hop2p Localizes to Meiotic Chromosomes

Anti-Hop2p antibodies were generated and used to localize the Hop2 protein by indirect immunofluorescence. Hop2p localizes to spread chromosomes from wild type, both prior to and during the period of Zip1p localization to chromosomes (Figures 7A–7C). This staining is specific for Hop2p, since it is not observed in a *hop2* null mutant (data not shown). At leptotene/zygotene, Hop2p staining appears somewhat punctate. The large number of Hop2p foci and the apparent overlap between foci precludes an accurate measurement of the number of foci per nucleus. By pachytene, Hop2p staining appears to be more intense and homogeneous, probably because chromosome condensation brings the Hop2p-staining closer together. As the SC disassembles, Hop2p dissociates from chromosomes (data not shown). Hop2p also localizes to chromosomes in a *spo11* mutant (Figures 7D–7E) that fails to initiate meiotic recombination.

Discussion

The *hop2* null mutation confers a novel phenotype. Chromosomes undergo extensive SC formation, but most synapsis involves nonhomologous partners. This phenotype suggests that the Hop2 protein normally serves to prevent synapsis from initiating at inappropriate locations. The need for such a function is critical given the many dispersed repeated sequences present in the eukaryotic genome and the genetic evidence indicating that ectopic repeats can interact with each other during meiotic prophase.

In addition to the perturbation of synapsis, *hop2* cells are defective in recombination. The mutant sustains a wild-type level of DSBs, but these breaks remain unrepaired. The failure to produce mature recombinants in the *hop2* mutant might reflect a direct involvement of Hop2p in DSB repair (e.g., in strand invasion). However, for a number of reasons, we favor the view that the *hop2* defect in recombination is an indirect consequence of the failure of pairing and/or synapsis between homologs. First, the Hop2 protein localizes to chromosomes even in the absence of DSBs, unlike proteins presumed to be directly involved in recombination (i.e., Rad51p, Dmc1p, and Msh4p) (Bishop, 1994; J. Novak and G. S. R., unpublished data). Second, Hop2p does not colocalize with Dmc1p (data not shown), though both *hop2* and *dmc1* accumulate processed DSBs. Third, a direct involvement in DSB repair would not account for the *hop2* defect in homolog pairing and synapsis. Like *hop2*, the *dmc1* and *rad51* mutants accumulate DSBs with single-stranded tails (Bishop et al., 1992; Shinohara et al., 1992), yet almost all chromosomes in *dmc1* and *rad51* strains synapse with their homologs (see above;

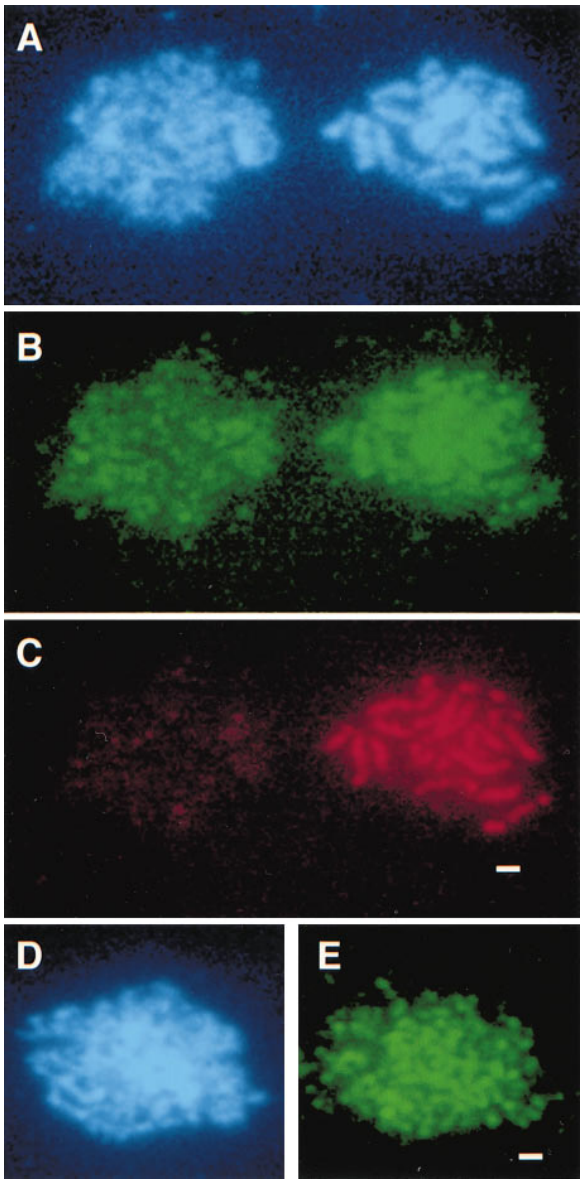


Figure 7. The Hop2 Protein Localizes to Meiotic Chromosomes

(A, B, and C) Wild-type (YAB36) spread nuclei at zygotene (left) and pachytene (right) stained with (A) DAPI (blue), (B) anti-Hop2p (green), and (C) anti-Zip1p (red) antibodies. (D and E) Nucleus from the *spo11* mutant (YAB88) stained with (D) DAPI (blue) and (E) anti-Hop2p antibodies (green). Scale bar = 1 μm.

Rockmill et al., 1995; unpublished data). The failure to repair DSBs in the *hop2* mutant may reflect the operation of a checkpoint that arrests the cell cycle prior to the completion of recombination (Lydall et al., 1996). This checkpoint may be triggered by the aberrant synapsis that occurs and/or by the failure of many DSBs to find homologous nonsister chromatids to use as substrates for repair.

Mutants exhibiting nonhomologous synapsis have been observed in a number of plants and animals. The mutant that appears most similar to *hop2* is found in *Allium fistulosum* (Jenkins and Okumus, 1992). In this mutant plant, nearly 80% of the axial element length is incorporated into SC, but synapsis occurs without regard to homology. Synapsed chromosomes exhibit fold-back loops and frequent changes of synaptic partners. The *Ph1* gene of wheat (reviewed by Sears, 1976) may perform a function similar to that of *HOP2*. The wheat genome is hexaploid, containing diploid sets of chromosomes from three closely related species. During meiosis in wild type, homologous chromosomes synapse with each other, but there is no synapsis between homeologous chromosomes from different chromosome sets. However, in the absence of *Ph1* gene function, chromosomes synapse with both homologs and homeologs, leading to multivalents instead of bivalents.

The *hop2* mutant is distinctly different from other *S. cerevisiae* mutants characterized to date. The *mre11S* mutant undergoes a limited amount of SC formation, and a number of observations suggest that some of the SCs formed involve nonhomologous chromosomes (Nairz and Klein, 1997). *mre11S* strains sustain the wild-type number of DSBs, but these breaks are not processed to expose single-stranded tails (Nairz and Klein, 1997). Thus, *hop2* differs from *mre11S* in two respects: *hop2* strains undergo DSB processing, and they assemble nearly wild-type amounts of SC. Like *dmc1* and *rad51*, *hop2* accumulates processed DSBs, but cytological analyses indicate that the *dmc1* and *rad51* mutants undergo little or no nonhomologous synapsis. This conclusion is based on three observations: chromosomes in *dmc1* and *rad51* strains are not difficult to separate during spreading (Figure 3B, Rockmill et al., 1995), *dmc1* and *rad51* undergo higher levels of homolog pairing than *hop2* (Table 1, Rockmill et al., 1995), and most unpaired chromosomes in *dmc1* (and presumably *rad51*) strains are not engaged in synapsis (see Results).

The existence of mutants in which chromosomes synapse, though they fail to pair, confirms that pairing and synapsis are distinct processes and that synapsis need not take place between homologous sequences. The latter conclusion is also supported by observations of extensive SC formation in haploid cells of yeast (Loidl et al., 1991) and plants (reviewed by von Wettstein et al., 1984). SC formation in organisms carrying chromosome rearrangements (e.g., inversions, deletions, and duplications) provides additional evidence that synapsis need not involve homologous chromosome segments. For example, in mice and *Neurospora* heterozygous for a large inversion (Moses et al., 1982; Bojko, 1990), an SC containing an inversion loop is evident at early pachytene. However, by late pachytene, the inversion loop is replaced by a linear SC in which the inverted region is heterosynapsed. This phenomenon of synaptic adjustment suggests that synapsis depends on homology at an early stage, but the requirement for homology is relaxed as meiosis progresses.

Previous studies have indicated that the processes of DSB repair and SC formation are intimately related. Mutants that fail to make DSBs fail to assemble SC (reviewed by Roeder, 1995, 1997). Even in haploids, which undergo nonhomologous synapsis, SC formation requires DSBs (unpublished data cited by Nairz and Klein, 1997). Mutant diploids that accumulate unprocessed DSBs undergo a limited amount of synapsis, at

least some of which is nonhomologous (Nairz and Klein, 1997). In the *dmc1* and *rad51* mutants, DSBs are processed to expose single-stranded tails, and nearly wild-type amounts of SC are assembled between homologs though synapsis is delayed (Rockmill et al., 1995). The Zip2 protein, which is involved in the initiation of synapsis, fails to localize to chromosomes in the absence of DSBs (Chua and Roeder, 1998); in a *rad50S* mutant, Zip2p colocalizes with proteins involved in DSB formation and processing (Chua and Roeder, 1998). Together, these observations suggest that synapsis initiates at DSB sites (Chua and Roeder, 1998). DSBs need not be processed for a limited amount of synapsis to occur, but processing appears to be necessary to ensure synapsis between homologs (Nairz and Klein, 1997). In the *hop2* mutant, DSBs are processed, but synapsis nevertheless takes place between nonhomologous chromosomes. Thus, single-stranded tails are not sufficient to ensure synapsis between homologs, though they may be necessary.

How does Hop2p ensure synapsis between homologs? We favor the view that Hop2p acts as a molecular "policeman" to prevent synapsis at inappropriate sites. The yeast genome contains naturally occurring dispersed repeated sequences, such as transposable elements. The high frequencies of ectopic recombination between artificial repeats during meiosis in wild type suggests that dispersed sequences are able to interact with each other during meiotic prophase. Hop2p may prevent synapsis from initiating at sites of ectopic interaction. The distinction between ectopic and genuine allelic interactions might involve an assessment of the degree of homology, with Hop2p recognizing when two sequences are imperfect homologs. In this case, Hop2p might perform a function similar to the MutS family of proteins involved in mismatch recognition. Alternatively, the distinction between allelic and ectopic homologies might involve a measure of the overall length of homology, with only long-range homology serving to satisfy Hop2p that homologs have been found. Another possible function for Hop2p is suggested by observations of short stretches of SC between nonhomologous chromosomes during zygotene in wild-type plants (reviewed by Carpenter, 1987). These stretches of ectopic synapsis are dissolved as meiosis progresses, and homologs become synapsed along their lengths. Perhaps Hop2p is involved in SC disassembly at ectopic sites.

If the *hop2* mutant initiates synapsis at the sites of dispersed repeats, then a *hop2* mutation might increase the commitment to ectopic gene conversion. To address this possibility, gene conversion was measured in strains carrying two mutant alleles of the *ARG4* gene inserted at different locations in the genome (data not shown), as described by Goldman and Lichten (1996). As expected, the *hop2* mutation decreased allelic recombination (an average of 4-fold) but increased ectopic recombination (an average of 5-fold). However, we cannot be certain of the extent to which the substrates for DSB repair were selected after the return to growth medium.

An alternative view of Hop2p function is that the protein actively participates in the alignment of homologous chromosomes. For example, Hop2p may be involved in the homology search process, comparing sequences one against another, in order to find identity. As noted above, none of the RecA homologs in yeast are required for homolog pairing (Kleckner, 1996). Perhaps Hop2p, though not a homolog of known strand exchange enzymes, is able to catalyze the formation of paranemic joints. Alternatively, Hop2p may stabilize pairing interactions promoted by other (as yet unknown) proteins. It is unlikely that Hop2p participates in telomere-mediated pairing (Rockmill and Roeder, 1998), since the protein is not concentrated at chromosome ends. Instead, chromosome ends may bring homologs into close physical proximity (reviewed by Dernburg et al., 1995), thus increasing the probability that proteins involved in homology searching (possibly including Hop2p) will meet with success.

Indiscriminate synapsis is not an inevitable consequence of a defect in meiotic interhomolog interactions. Of the many yeast meiotic mutants characterized that perturb some aspect of pairing, recombination, or synapsis (reviewed by Roeder, 1995, 1997), *hop2* is unusual in displaying extensive synapsis between nonhomologous chromosomes. This surprising aspect of the *hop2* mutant phenotype argues that the wild-type Hop2 protein functions specifically to prevent the initiation of SC formation at inappropriate sites. Future studies of *HOP2* are expected to provide insight into the mechanisms that prevent indiscriminate synapsis and thereby ensure synapsis between homologs.

Experimental Procedures

Yeast Strains and Genetic Procedures

Yeast strain genotypes are listed in Table 3. Substitutive and integrative transformations (Rothstein, 1991) were carried out by the lithium acetate procedure (Ito et al., 1983). YAB7, YAB15, YAB24, YAB27, YAB36, YAB85, YAB86, YAB87, YAB88, YAB89, YAB92, and YAB125 are isogenic and derived from mating BR1373-6D (Rockmill and Roeder, 1990), or a transformant thereof, to YB-1, or a transformant thereof. All SYAB diploids are isogenic with the rapidly sporulating strain SK1 (Cao et al., 1990). Strains YAB45, YAB46, YAB49, and YAB51 are isogenic and derived by mating RLK1-3C, or a transformant thereof, to K144, or a transformant thereof (Voelkel-Meiman and Roeder, 1990).

To sporulate cells for cytological analyses and physical assays of recombination, cells were pregrown in YPA (Bishop et al., 1992) to a concentration of $\sim 2 \times 10^7$ cells/ml and then washed and resuspended in 2% KAc. Sporulation was carried out at 30° with vigorous shaking.

Isolation of the *hop2-1* Mutant and Cloning of the *HOP2* Gene and cDNA

Strain CY32A2 was mutagenized with ultraviolet light to 50% killing, and survivors were screened for defects in meiotic gene conversion as described (Menees and Roeder, 1989). PC72 was identified as a mutant in which gene conversion is decreased compared to wild type. A *MAT*α derivative of PC72 was isolated by treatment with 100 μg/ml methyl benzimidazole-2-yl carbonate (Aldrich Chemical Co.) to induce chromosome loss, followed by selection on medium containing 2 mg/l cryptopleurine (Chemasea), which demands expression of the recessive cryptopleurine-resistance mutation linked to *MAT*α.

The *HOP2* gene was isolated from a yeast genomic library described previously (Menees and Roeder, 1989). Transformants of a diploid homozygous for *hop2-1* (PC72ab) were selected on medium lacking leucine, replica plated to sporulation medium, and screened for sporulation using the ether test (Rockmill and Roeder, 1988).

To prepare *HOP2* cDNA, yeast RNA was isolated as described by Menees et al. (1992). *HOP2* mRNA was reverse transcribed, and

Table 3. Yeast Strains

Strain	Genotype
CY32A2	*MAT***a** *CRY1 CEN3(TRP1) leu2-3 his4-280 spo13::ADE2 trp1 ura3-1 ade2-1* *MATα cry1 CEN3 leu2-3,112 his4-260*
PC72	CY32A2 but *hop2-1*
PC72ab	*MAT***a** *CRY1 leu2-27 his4-280 trp1-1 spo13::ADE2 lys2 ade2-1 hop2-1* *MATα cry1 leu2-112 his4-260 trp1-H3 spo13::ADE2 LYS2 ade2-1 hop2-1*
BR2171-7B	*MAT***a** *leu2 ura3-1 HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1* *MATαleu2 ura3-1 HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1*
BR2495	*Mat***a** *leu2-27 his4-280 trp1-1 arg4-8 thr1-4 ura3-1 ade2-1* *MATα leu2-112 his4-260 trp1-289 ARG4 thr1-1 ura3-1 ade2-1*
YAB01	BR2495 but homozygous *hop2::LEU2*
BR1373-6D	*MAT***a** *leu2-27 his4-280 ura3-1 spo13::ura3-1 arg4-8 thr1-1 trp1-1 ade2-1 cyh10^r*
YB-1	*MATα leu2-3,112 his4-260 ura3-1 trp1-289 spo13::ura3-1 arg4-9 thr1-4 lys2-1 ade2-1*
YAB36	*MAT***a** *Leu2-27 his4-280 ura3-1 trp1-1 spo13::ura3-1 arg4-9 thr1-4* *MATα leu2-3,112 his4-260 ura3-1 trp1-289 SPO13 arg4-8 thr1-1* *cyh10 LYS2 ade2-1* *CYH10 lys2-1 ade2-1*
YAB7	YAB36 but homozygous *spo13::ura3-1*
YAB15	YAB36 but homozygous *spo13::ura3-1 hop2::LEU2*
YAB24	YAB36 but homozygous *spo13::ura3-1 hop2:URA3*
YAB27	YAB36 but homozygous *hop2::URA3*
YAB85	YAB36 but homozygous *spo13::ura3-1 hop2::URA3 spo11::TRP1*
YAB86	YAB36 but homozygous *hop2::URA3 spo11::TRP1*
YAB87	YAB36 but homozygous *spo13::ura3-1 spo11::TRP1*
YAB88	YAB36 but homozygous *spo11::TRP1*
YAB89	YAB36 but homozygous *zip1::LEU2*
YAB92	YAB36 but homozygous *hop2::URA3 zip1::LEU2*
YAB125	YAB36 but homozygous *dmc1::LEU2*
SYAB1	*MAT***a** *leu2-hisG his4-X-LEU2-URA3 ura3 arg4-nsp-URA3* *MATα leu2-hisG his4-B-LEU2 ura3 arg4-bgl*
SYAB2	SYAB1 but homozygous *hop2::URA3*
SYAB3	SYAB1 but hoozygous *rad50-K181::URA3 hop2::URA3*
SYAB4	*MAT***a** *leu2-hisG his4-X-LEU2 ura3 arg4-nsp-URA3 rad50-K181::URA3* *MATα leu2-hisG his4-B-LEU2 ura3 arg4-bgl rad50-K181::URA3*
SYAB5	SYAB4 but homozygous *hop2::URA3*
SYAB6	*MAT***a** *leu2-R ura3 arg4-nsp* *MATα leu2-K ura3 arg4-bgl*
SYAB7	SYAB6 but homozygous *hop2::LEU2*
SYAB8	SYAB6 but *hop2::lacZ* *HOP2*
SYAB9	SYAB6 but *red1::lacZ* *RED1*
YAB45	*MAT***a** *his4-712 ura3-52 ADE2-BIK1-ade2* *MATα his4-Hpa ura3-52 ADE2-BIK1-ade2*
YAB46	YAB45 but homozygous *hop2::URA3*
YAB49	YAB45 but *his4-712/his-260* and homozygous *hop2::URA3*
YAB51	YAB45 but *his4-712/his-260*

The allele referred to as *rad50-K181::URA3* in SYAB3 and SYAB4 is referred to as *rad50S* elsewhere in the text.

the resulting cDNA was amplified by PCR using the GeneAmp Thermostable rTth Reverse Transcriptase RNA PCR kit (Perkin-Elmer Cetus). The upstream primer was 5′-GAACTGACCAAGCTTTATTTG AAAGATATG-3′ (located at positions −27 to +3 relative to the numbering system indicated in Figure 1A), and the downstream primer was 5′-GGTCTCGAAAAGCTTCAAAATACATACCA-3′ (located at positions +688 to +717 in the *HOP2* gene, Figure 1A). The PCR product was purified and subcloned into pBS M13+ (Stratagene) prior to sequencing.

Plasmids

pL13 was constructed by inserting a 2.2 kb HindIII-SphI fragment containing the entire *HOP2* gene from pL1 into pUN100 (Elledge and Davis, 1988). pL14 and pL19 were constructed by inserting the 2.2 kb HindIII-PstI fragment containing *HOP2* (from pL13) into pHSS6 (Hoekstra et al., 1991) and pBS M13+ (Stratagene), respectively. To construct pL16, the PstI-TaqI fragment from pL14 was inserted between the PstI and ClaI sites of the pHSS6 polylinker.

pL14 was subjected to transposon mutagenesis using m-Tn3 (*LEU2 lac*) (Hoekstra et al., 1991). Two of the resulting mutagenized plasmids, pL14-Tn12 and pL14-Tn24, were used to construct the *hop2::LEU2* deletion/disruption mutation. To do so, both pL14-Tn12 and pL14-Tn24 were digested with HindIII and NdeI (which cuts in the transposon). The upstream HindIII-NdeI fragment from pL14-Tn12 was ligated to the downstream HindIII-NdeI fragment from pL14-Tn24, resulting in deletion of *HOP2* sequences between the two transposons and retention of the *LEU2*-marked transposon. The resulting plasmid, pL15, was cut with NotI prior to yeast transformation. The *hop2::LEU2* mutation removes nucleotides +271 to +593 of the *HOP2* gene (Figure 1A).

The *hop2::URA3* allele was constructed by replacing the 0.7 kb BglII-NheI fragment of pL19 with a 1.5 kb SspI-NheI fragment containing the *URA3* gene. The resulting plasmid, pL21, was cut with XbaI prior to yeast transformation. The *hop2::URA3* deletion removes nucleotides −253 to +456 of the *HOP2* gene (Figure 1A).

pL22, containing the *GST-HOP2* fusion gene, was constructed by ligating the XmaI fragment of pL16 into the XmaI site of the expression vector pGEX-3X (Smith and Johnson, 1988). The fusion gene contains nucleotides +108 to +713 from the *HOP2* gene (Figure 1A).

pL115, pL116, and pL117 were used to generate chromosome VII

probes for FISH analysis. All three plasmids were derived by inserting HindIII fragments from *hop2*-complementing clones into the HindIII site of pBS M13− (Stratagene). pL115 carries the ~6 kb HindIII fragment containing *HOP2*; pL116 contains a ~6 kb fragment located immediately downstream, and pL117 contains a ~4 kb fragment located immediately upstream.

The *zip1::LEU2*, *red1::lacZ*, and *spo11::TRP1* mutations were introduced by transformation with pR1560 (Sym et al., 1993), pB69 (Rockmill and Roeder, 1988), and pGB324 (obtained from Craig Giroux), respectively.

β-Galactosidase Assays

For assays of gene expression, cells were grown and sporulated as described above, except that cells were grown in YPA to a concentration of 5×10^6 cells/ml prior to sporulation. β-galactosidase assays were performed as described (Chua and Roeder, 1998). Values given in Figure 1 are the averages of two independent cultures for each strain. β-galactosidase units are defined as nanomoles of o-nitrophenyl-β-D-galactopyranoside cleaved per minute per milligram of protein.

Physical Assays of Recombination

Zeta-probe nylon membrane (Bio-Rad) was used for Southern blotting. ^{32}P labeling was carried out according to the Prime-a-Gene kit (Promega). DSB frequencies were quantitated by scanning autoradiograms and analyzing the resulting data using Multi-Analyst software (Bio-Rad).

Antibodies

Rabbit anti-Dmc1p antibody was provided by Douglas Bishop. Rabbit and mouse anti-Hop2p antibodies were raised against the GST-Hop2 fusion protein encoded by pL22. Rabbit and mouse anti-Zip1p antibodies were raised against the GST-Zip1p fusion protein encoded by pMB114 (Sym et al., 1993). Anti-Zip1p and anti-Hop2p antibodies were generated at the Pocono Rabbit Farm and Laboratory. Anti-Zip1p and anti-Hop2p antibodies were affinity purified as described by Chua and Roeder (1998). Secondary antibodies were conjugated with Texas red or fluorescein isothiocyanate (Jackson Immuno Research Laboratories, Inc.).

Cytology

Meiotic chromosomes were prepared for electron and fluorescence microscopy according to the procedures of Loidl et al. (1991). For FISH analyses, chromosomes were spread according to Dresser and Giroux (1988). Immunofluorescence was performed as described by Sym et al. (1993). A Leica DMRB microscope was used to observe antibody-stained preparations. Images were captured using a Photometrics Imagepoint CCD camera and Imagepoint Lab Spectrum software (Signal Analytics Corporation).

To quantitate the intensity of anti-Zip1p staining, images of nuclear spreads were captured by a CCD camera. The region of a whole nuclear spread (excluding polycomplex) was outlined; the pixel number within each spread was then counted, and the background pixel number was subtracted. Data analysis was performed using Imagepoint Lab Spectrum software.

To examine cell division, cells harvested at different time points after the induction of meiosis were fixed, stained with DAPI, and examined in the fluorescence microscope as described (Thompson and Roeder, 1989). At each time point, 500 cells were counted for each strain examined.

FISH was performed as described by Chua and Roeder (1998). The chromosome VII probe was derived from pL115, pL116, and pL117; see Chua and Roeder (1998) for chromosome III and V probes. For FISH and anti-Zip1p staining, anti-Zip1p staining was performed as described above, and slides were fixed in 3.7% formaldehyde for 20 min. After washing, FISH was carried out.

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References

Alani, E., Padmore, R., and Kleckner, N. (1990). Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell *61*, 419–436.

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.

Bojko, M. (1990). Synaptic adjustment of inversion loops in *Neurospora crassa*. Genetics *124*, 593–598.

Cao, L., Alani, E., and Kleckner, N. (1990). A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell *61*, 1089–1101.

Carpenter, A.T.C. (1987). Gene conversion, recombination nodules, and the initiation of meiotic synapsis. BioEssays *6*, 232–236.

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

Dernburg, A.F., Sedat, J.W., Cande, W.Z., and Bass, H.W. (1995). Cytology of telomeres. In Telomeres, E.H. Blackburn and C.W. Grieder, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 295–337.

Dresser, M.E., and Giroux, C.N. (1988). Meiotic chromosome behavior in spread preparations of yeast. J. Cell Biol. *106*, 567–578.

Elledge, S.J., and Davis, R.W. (1988). A family of versatile centromeric vectors designed for use in the sectoring-shuffle mutagenesis assay in *Saccharomyces cerevisiae*. Gene *70*, 303–312.

Goldman, A.S.H., and Lichten, M. (1996). The efficiency of meiotic recombination between dispersed sequences in *Saccharomyces cerevisiae* depends upon their chromosomal location. Genetics *144*, 43–55.

Goyon, C., and Lichten, M. (1993). Timing of molecular events in meiosis in *Saccharomyces cerevisiae*: stable heteroduplex DNA is formed late in meiotic prophase. Mol. Cell. Biol. *13*, 373–382.

Hoekstra, M.F., Seifert, H.S., Nickoloff, J., and Heffron, F. (1991). Shuttle mutagenesis: bacterial transposons for genetic manipulations in yeast. Methods Enzymol. *194*, 329–369.

Ito, H., Fukada, Y., Murata, K., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. *153*, 163–168.

Jenkins, G., and Okumus, A. (1992). Indiscriminate synapsis in achiasmate *Allium fistulosum* L. (Liliaceae). J. Cell Sci. *103*, 415–422.

Jinks-Robertson, S., Sayeed, S., and Murphy, T. (1997). Meiotic crossing over between nonhomologous chromosomes affects chromosome segregation in yeast. Genetics *146*, 69–78.

Kleckner, N. (1996). Meiosis: how could it work? Proc. Natl. Acad. Sci. USA *93*, 8167–8174.

Loidl, J., Nairz, K., and Klein, F. (1991). Meiotic chromosome synapsis in a haploid yeast. Chromosoma *100*, 221–228.

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.

Lydall, D., Nikolsky, Y., Bishop, D.K., and Weinert, T. (1996). A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature *383*, 840–843.

Menees, T.M., and Roeder, G.S. (1989). *MEI4*, a yeast gene required for meiotic recombination. Genetics *123*, 675–682.

Menees, T.M., Ross-Macdonald, P.B., and Roeder, G.S. (1992).

MEI4, a meiosis-specific yeast gene required for chromosome synapsis. Mol. Cell. Biol. *12*, 1340–1351.

Mitchell, A.P. (1994). Control of meiotic gene expression in *Saccharomyces cerevisiae*. Microbiol. Rev. *58*, 56–70.

Moses, M.J., Poorman, P.A., Roderick, T.H., and Davisson, M.T. (1982). Synaptonemal complex analysis of mouse chromosomal rearrangements. IV. Synapsis and synaptic adjustment in two paracentric inversions. Chromosoma *84*, 457–474.

Nairz, K., and Klein, F. (1997). *mre11S*—a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev. *11*, 2272–2290.

Parker, D.R. (1969). Heterologous interchange at meiosis in Drosophila. II. Some disjunctional consequences of interchange. Mutat. Res. *7*, 393–407.

Petes, T.D., Malone, R.E., and Symington, L.S. (1991). Recombination in yeast. In The Molecular and Cellular Biology of the Yeast *Saccharomyces*: Genome Dynamics, Protein Synthesis, and Energetics, J.R. Broach, J.R. Pringle, and E.W. Jones, eds. (Plainview, NY: Cold Spring Harbor Laboratory Press), pp. 407–521.

Prinz, S., Amon, A., and Klein, F. (1997). Isolation of *COM1*, a new gene required to complete meiotic double-strand break induced recombination in *Saccharomyces cerevisiae*. Genetics *146*, 781–795.

Rockmill, B., and Roeder, G.S. (1988). *RED1*: a yeast gene required for the segregation of chromosomes during the reductional division of meiosis. Proc. Natl. Acad. Sci. USA *85*, 6057–6061.

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., in press.

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. (1995). Sex and the single cell: meiosis in yeast. Proc. Natl. Acad. Sci. USA *92*, 10450–10456.

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

Rothstein, R. (1991). Targeting, disruption, replacement and allele rescue: integrative DNA transformation in yeast. Methods Enzymol. *194*, 281–301.

Sears, E.R. (1976). Genetic control of chromosome pairing in wheat. Annu. Rev. Genet. *10*, 31–51.

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.

Smith, D.B., and Johnson, K.S. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene *67*, 31–40.

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., and Roeder, G.S. (1995). Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. J. Cell Biol. *128*, 455–466.

Sym, M., Engebrecht, J., and Roeder, G.S. (1993). ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell *72*, 365–378.

Tettelin, H., Carbone, M.L.A., Albermann, K., Albers, M., Arroyo, J., Backes, U., Barreiros, T., Bertani, I., Bjourson, A.J., Brückner, M., et al. (1997). The nucleotide sequence of *Saccharomyces cerevisiae* chromosome VII. Nature *387 (6632 Suppl.)*, 81–84.

Thompson, E.A., and Roeder, G.S. (1989). Expression and DNA sequence of *RED1*, a gene required for meiosis I chromosome segregation in yeast. Mol. Gen. Genet. *218*, 293–301.

Tung, K.S., and Roeder, G.S. (1998). Meiotic chromosome morphology and behavior in *zip1* mutants of *Saccharomyces cerevisiae*. Genetics *149*, 817–832.

Voelkel-Meiman, K., and Roeder, G.S. (1990). A chromosome containing HOT1 preferentially receives information during mitotic interchromosomal gene conversion. Genetics *124*, 561–572.

von Wettstein, D., Rasmussen, S.W., and Holm, P.B. (1984). The synaptonemal complex in genetic segregation. Annu. Rev. Genet. *18*, 331–413.

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

West, S.C. (1992). Enzymes and molecular mechanisms of genetic recombination. Annu. Rev. Biochem. *61*, 603–640.

GenBank Accession Number

The accession number for the *HOP2* gene is AF078740.