Heteroduplex DNA Formation and Homolog Pairing in Yeast Meiotic Mutants

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ABSTRACT

Previous studies of Saccharomyces cerevisiae have identified several meiosis-specific genes whose products are required for wild-type levels of meiotic recombination and for normal synaptonemal complex (SC) formation. Several of these mutants were examined in a physical assay designed to detect heteroduplex DNA (hDNA) intermediates in meiotic recombination. hDNA was not detected in the rec102, mer4 and hop1 mutants; it was observed at reduced levels in red1, mekl and mer1 strains and at greater than the wild-type level in siipl. These results indicate that the REC102, MER4, HOP1, RED1, MEK1 and MER1 gene products act before hDNA formation in the meiotic recombination pathway, whereas ZIP1 acts later. The same mutants assayed for hDNA formation were monitored for meiotic chromosome pairing by in situ hybridization of chromosome-specific DNA probes to spread meiotic nuclei. Homolog pairing occurs at wild-type levels in the siipl and mekl mutants, but is substantially reduced in mer4, rec102, hop1, red1 and mer1 strains. Even mutants that fail to recombine or to make any SC or SC precursors undergo a significant amount of meiotic chromosome pairing. The in situ hybridization procedure revealed defects in meiotic chromatin condensation in mer1, red1 and hop1 strains.

Genetic analysis in fungi has provided insight into the molecular mechanisms of meiotic recombination (ORR-WEAVER and SZOSTAK 1985; PETES et al. 1991). Meiotic genetic exchange involves DNA strand transfer to create hybrid DNA, consisting of one strand from each of the two recombining DNA duplexes. If the participating DNA molecules are genetically different within the region of strand exchange, the hybrid DNA contains one or more mismatched base pairs and is referred to as heteroduplex DNA (hDNA). Repair of a mismatched base pair in the appropriate direction results in gene conversion (6:2 or 2:6 segregation). A failure to repair results in postmeiotic segregation (5:3 or 3:5 segregation). Approximately 50% of aberrant segregation events (including both gene conversion and postmeiotic segregation) are accompanied by reciprocal crossing over between flanking markers, leading to models in which aberrant segregation with or without crossing over represent alternative modes of resolution of a common intermediate (ORR-WEAVER and SZOSTAK 1985).

In recent years, a variety of physical assays have been designed to detect recombination intermediates and recombinant products generated during meiosis in yeast. Assays have been developed that detect double-strand breaks (GAME et al. 1989; SUN et al. 1989; CAO et al. 1990; ZENVIRTH et al. 1992), single-stranded tails (SUN et al. 1991; BISHOP et al. 1992), joint molecules (COLLINS and NEWLON 1994; SCHWACHA and KLECKNER 1994), hDNA (LICHTEN et al. 1990; NAG and PETES 1993), and crossover products (BORTS et al. 1986; PADMORE et al. 1991; GÖYON and LICHTEN 1993; NAG and PETES 1993). Physical assays have shed light on the molecular mechanisms of meiotic recombination; e.g., they led to the demonstration that most or all meiotic recombination events in yeast are initiated by double-strand breaks (SUN et al. 1989; CAO et al. 1990; ZENVIRTH et al. 1992; WU and LICHTEN 1994). In addition, physical assays have made it possible to monitor recombination in mutants that fail to sporulate and in those that sporulate, but produce inviable products (BORTS et al. 1986; BISHOP et al. 1992; SHINOHARA et al. 1992; SYM et al. 1993). Finally, physical assays have facilitated studies of the relationship between meiotic recombination and chromosome synopsis (PADMORE et al. 1991).

The alignment of homologous chromosomes during meiotic prophase culminates in the formation of the synaptonemal complex (SC). The SC consists of two parallel lateral elements and an intervening central region. Each lateral element represents the protein backbone of one pair of condensed sister chromatids (VON WETTSTEIN et al. 1984) and is called an axial element before homologs synapse. The lateral elements are connected by transverse filaments that lie perpendicular to the long axis of the complex and span the central region. Temporal studies have demonstrated that meiotic recombination and SC assembly proceed concurrently.
(PADMORE et al. 1991). Double-strand breaks with single-stranded tails occur early in prophase, before the formation of tripartite SC (PADMORE et al. 1991). Double-strand breaks disappear during zygotene, when synopsis initiates and the SC begins to zipper up (PADMORE et al. 1991). During pachytene, when chromosomes are fully synapsed, joint molecules that contain DNA from both parental chromosomes are evident (COLLINS and NEWLON 1994; SCHWACHA and KLECKNER 1994). Crossover products are detected at the end of, or just after, pachytene as the SC disassembles (PADMORE et al. 1991) and hDNA is detected at the same time, or slightly earlier (GOVON and LIGHTEN 1993; NAG and PETES 1993; SCHWACHA and KLECKNER 1994).

The formation of mature SC is preceded by an homology search that results in the side-by-side alignment of homologs at a distance greater than the width of the SC (LOIDL. 1990). In some organisms, the parallel arrangement of axial elements can be visualized in silver-stained preparations. In yeast, however, chromosome pairing before pachytene cannot be visualized by silver staining because axial elements are not fully developed at the relevant time in meiotic prophase (PADMORE et al. 1991). To detect homolog pairing, the technique of fluorescent in situ hybridization (FISH) has been applied to spread preparations of meiotic chromosomes (SCHERTHAIN et al. 1992; WEINER and KLECKNER 1994). Using this method in conjunction with composite, chromosome-specific DNA probes, it has been possible to detect presynaptic alignment in wild-type yeast (SCHERTHAIN et al. 1992). FISH has also been used to assess homolog pairing in vegetative cells and in meiotic mutants that fail to make SC (LOIDL et al. 1994; WEINER and KLECKNER 1994). The results indicate that homologs are paired in a substantial fraction of cells just before entry into meiosis (LOIDL et al. 1994; WEINER and KLECKNER 1994). Chromosomes become unpaired during premeiotic DNA replication; then pairing is reestablished, independent of SC (WEINER and KLECKNER 1994).

In Saccharomyces cerevisiae, numerous mutants defective in meiotic recombination and chromosome synapsis have been isolated and characterized. In this paper, we have assayed hDNA formation and homolog pairing in strains carrying null mutations in seven different genes that are expressed and function specifically in meiotic cells. Our results provide insight into the steps in meiosis at which the MEH4, REC102, HOP1, RED1, MEK1, MERI and ZIP1 gene products act.

**MATERIALS AND METHODS**

**Yeast strains and plasmids:** Meiotic-lethal mutations were introduced into haploid yeast strains using the LiAc transformation procedure (ROSE et al. 1990). The hop1::TRP1 allele was introduced by two-step transplacement; all other meiotic genes were disrupted in one step (ROTHSTEIN 1991). The plasmids used to introduce meiotic mutations and the enzymes with which the plasmids were digested prior to transformation into yeast are listed in Table 1.

All strains used for hDNA analysis were isogenic derivatives of the haploid strains AS4 and AS15 (NAG et al. 1989). These haploids were transformed and then mated to generate the DNY diploids listed in Table 2. Plasmids and strategies used to introduce the hix-hIR15, his-hIR16 and leu-hIR16 mutants were described previously (NAG and PETES 1993). In all DNY strains except DNY163, the URA3 gene was inserted just downstream of the hix-hIR15 allele using pMW33 (NAG and PETES 1993) cut with Asp718. In DNY163, the Spel site downstream of HIS4 was destroyed by two-step transplacement using pMB1 targeted with SnaBl pMB1, provided by Monika Wierdel, contains the BglII fragment downstream of the HIS4 gene inserted at the BamHI site of YEp; the Spel site downstream of HIS4 has been filled in.

All strains used for FISH were derived from the haploids BR1373-6D and BR1919-8B (ROCKMILL and ROEDER 1990); these haploids were mated to generate BR2495. Strains S1574, JB278 (BHARGAVA et al. 1991), BR2498, BR2486, BR2496 (ENGEBRECHT et al. 1991), BR2626 (ROCKMILL and ROEDER 1991) and MY63 (SM et al. 1993) were generated by mating transformants of BR1373-6D to transformants of BR1919-8B.

**Physical analysis of hDNA:** Meiosis was induced, meiotic DNA was isolated and hDNA was monitored as described by NAG and PETES (1993). DNA harvested from 15 ml of sporulating cells was dissolved in 80–100 μl. Six μl of the DNA solution from each time point was digested and analyzed by Southern blotting using a HIS4-specific DNA probe (the XbaI-XhoI fragment of pDN42) (NAG and PETES 1995). The amount of hDNA formed at different time points was quantitated using a Molecular Dynamics phosphorimager.

**FISH:** Homolog pairing was assessed by FISH to spread chromosomes from strain BR2495 and isogenic diploids. Cells were grown to saturation at 30°C in YEPD medium (ROSE et al. 1990) supplemented with adenine, then diluted 1:7.5 into 2% KAc and incubated at 30°C for 15 hr. Cells were spread as described by DRESSER and GIRoux (1988) and ENGEBRECHT and ROEDER (1990) with the following modifications: resuspended spheroplasts were poured onto clean glass slides and incubated for 15 min. FISH with chromosome-specific, composite DNA probes (chromosome painting) was carried out as described previously (SCHERTHAIN et al. 1992; LOIDL et al. 1994). The probe for painting chromosome I consists of four lambda clones representing ~60 kb of this 230-kb chromosome. The probe for painting chromosome III consists of 10 plasmid clones, containing 120 kb spanning 185 kb of this 340-kb chromosome. The chromosome I probe was labeled with digoxigenin-11-dUTP (Boehringer) and detected with mouse antidigoxigenin antibodies followed by anti-mouse IgG conjugated to tetramethylrhodamine isothiocyanate. The chromosome III probe was labeled with biotin-14-dATP (Life Technologies) and detected with avidin conjugated to fluorescein isothiocyanate. Homolog pairing was scored in nuclei in which both chromosome probes presented compact hybridization signals. In most such nuclei, individual chromosomes were evident as "sausages" stained with 4',6-diamidino-2-phenylindole (DAPI). Nuclei with this chromosome morphology were used to assess homolog pairing to minimize the contribution of unsporulated cells and of cells at stages of meiosis other than pachytene. In cells at metaphase of meiosis I, a spindle-shaped region of negative staining was evident in DAPI fluorescence. Such nuclei were excluded from measurements of homolog pairing.

The frequency of association between homologs was corrected for accidental associations as inferred from the frequency of association between heterologous chromosomes (LOIDL et al. 1994). The corrected frequencies for chromosomes I and III were averaged in each experiment. To com-
TABLE 1

<table>
<thead>
<tr>
<th>Gene disruption</th>
<th>Plasmid</th>
<th>Reference</th>
<th>Targeting enzyme(s)</th>
</tr>
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<tbody>
<tr>
<td>mei4::URA3</td>
<td>pTM6</td>
<td>MENENEES and ROEDER (1989)</td>
<td>Nod</td>
</tr>
<tr>
<td>mei4::ADE2</td>
<td>pTM7</td>
<td>MENENEES and ROEDER (1989)</td>
<td>Nod</td>
</tr>
<tr>
<td>rec102::LEU2</td>
<td>pJB7</td>
<td>BHRAGA et al. (1993)</td>
<td>Nod</td>
</tr>
<tr>
<td>hop1::LEU2</td>
<td>pNH37-2</td>
<td>HOLLINGSWORTH and BYERS (1989)</td>
<td>BglII</td>
</tr>
<tr>
<td>red1::LEU2</td>
<td>pB72</td>
<td>ROCKMILL and ROEDER (1988)</td>
<td>Nod</td>
</tr>
<tr>
<td>mek1::LEU2</td>
<td>pB84</td>
<td>ROCKMILL and ROEDER (1990)</td>
<td>Cid</td>
</tr>
<tr>
<td>mer1::LEU2</td>
<td>pME162</td>
<td>ENGBRECHT and ROEDER (1989)</td>
<td>SmaI</td>
</tr>
<tr>
<td>mek1::LYS2</td>
<td>pB121</td>
<td>ROCKMILL and ROEDER (1991)</td>
<td>Nod</td>
</tr>
<tr>
<td>zip1::LEU2</td>
<td>pMB97</td>
<td>SYM et al. (1993)</td>
<td>ApoI and SndIII</td>
</tr>
</tbody>
</table>

TABLE 2

<table>
<thead>
<tr>
<th>Yeast strains</th>
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<tbody>
<tr>
<td>Strain</td>
</tr>
<tr>
<td>DNY143</td>
</tr>
<tr>
<td>DNY163</td>
</tr>
<tr>
<td>DNY127</td>
</tr>
<tr>
<td>DNY162</td>
</tr>
<tr>
<td>DNY122</td>
</tr>
<tr>
<td>DNY169</td>
</tr>
<tr>
<td>DNY124</td>
</tr>
<tr>
<td>DNY154</td>
</tr>
<tr>
<td>BR2495</td>
</tr>
<tr>
<td>S1574</td>
</tr>
<tr>
<td>JB278</td>
</tr>
<tr>
<td>BR2498</td>
</tr>
<tr>
<td>BR2486</td>
</tr>
<tr>
<td>BR2496</td>
</tr>
<tr>
<td>BR2526</td>
</tr>
<tr>
<td>MY63</td>
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</tbody>
</table>
diploid heterozygous for a palindromic insertion at the SalI site is sporulated at 25°C, 20–25% of tetrads display postmeiotic segregation at HIS4 and ~70% of these (i.e., ~16% of total tetrads) show postmeiotic segregation (FAN et al. 1995). In the experiment reported in Figure 2A and Table 3, the maximum level of heteroduplex DNA was reached at 20 hr after introduction into sporulation medium. At this time point, 3.2% of total meiotic DNA was heteroduplex; this number corresponds reasonably well to the value expected if ~16% of the tetrads display postmeiotic segregation and one spore in each of these tetrads contains heteroduplex. At later time points, the amount of hDNA appears to decrease, presumably reflecting the difficulty in recovering DNA from spores. At late times, when many cells have sporulated, any remaining vegetative cells make a disproportionate contribution to the DNA recovered, leading to an underestimate of hDNA (NAG and PETES 1993).

**hDNA in meiotic mutants:** Derivatives of the wild-type strain, DNY143, were constructed that are homozygous for null mutations in genes required for wild-type levels of meiotic recombination. DNA was analyzed from cells collected early after meiotic induction and at late time points when hDNA formation in wild type had reached its maximum level. In the DNY strain background, all of the mutants tested sporulate with reduced efficiency (Table 3). In addition, all of the mutants display reduced spore viability (Table 3), consistent with previous studies (ENGEBRECHT and ROEDER 1989; HOLLINGSWORTH and BYERS 1989; MENEES and ROEDER 1989; ROCKMILL and ROEDER 1990, 1991; BHARGAVA et al. 1991; COOL and MALONE 1992; LEEM and OGAWA 1992; SYM and ROEDER 1994).

The *mer4, rec102* and *hop1* mutants failed to produce any detectable hDNA, even at late time points in meiosis (Figure 2, B and C, Table 3, data not shown). hDNA that occurs at ~5% of the wild-type level can be detected in this assay. The maximum amount of hDNA produced by the *red1, mekl* and *mer1* null mutants was 8, 20.5 and 14.5%, respectively, of the wild-type level (Figure 3, A–C, Table 3). The *zip1* null mutant produced almost twice (189%) as much hDNA as wild type (Figure 3D, Table 3). The time point at which the maximum level of hDNA was reached in the *zip1* mutant was later than in wild type in this and other experiments.

![Figure 1](https://academic.oup.com/genetics/article/141/1/75/6013492)

**Figure 1.**—Strand exchange between the *his4-IR15* and *his4-IR16* alleles. Thick lines indicate the wild-type chromosomal DNA and thin lines indicate the inserted palindromic sequences. DNA strands from nonsister chromatids are indicated by different shading. Brackets indicate the lengths of the restriction fragments generated after digestion with *BamHI, PstI* and *PvuII*. Figure is not drawn to scale.

![Figure 2](https://academic.oup.com/genetics/article/141/1/75/6013492)

**Figure 2.**—Physical analysis of hDNA formation in wild-type and *rec102* and *hop1* mutants. The number at the top of each lane represents the time (in hours) after introduction into sporulation medium. The sizes of restriction fragments are indicated to the right of each panel; the 2.4-kb band represents hDNA as indicated on the left. (A) DNY143 (wild type); (B) DNY127 (*rec102*); (C) DNY162 (*hop1)*.
TABLE 3
Heteroduplex formation in meiotic mutants

<table>
<thead>
<tr>
<th>Strain (relevant genotype)</th>
<th>Sporulation</th>
<th>Spore viability (%)</th>
<th>Total DNA in heteroduplex (%)</th>
<th>Wild-type heteroduplex (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNY143 (wild type)</td>
<td>35</td>
<td>86</td>
<td>3.2</td>
<td>100</td>
</tr>
<tr>
<td>DNY163 (mer1)</td>
<td>13</td>
<td>0</td>
<td>&lt;0.15</td>
<td>&lt;5</td>
</tr>
<tr>
<td>DNY127 (rec102)</td>
<td>24</td>
<td>0</td>
<td>&lt;0.15</td>
<td>&lt;5</td>
</tr>
<tr>
<td>DNY162 (hop1)</td>
<td>20</td>
<td>0</td>
<td>&lt;0.15</td>
<td>&lt;5</td>
</tr>
<tr>
<td>DNY122 (red1)</td>
<td>23</td>
<td>2.5</td>
<td>0.27</td>
<td>8</td>
</tr>
<tr>
<td>DNY169 (mer1)</td>
<td>3</td>
<td>0</td>
<td>0.48</td>
<td>14.5</td>
</tr>
<tr>
<td>DNY124 (mek1)</td>
<td>25</td>
<td>0</td>
<td>0.67</td>
<td>20.5</td>
</tr>
<tr>
<td>DNY154 (zip1)</td>
<td>9</td>
<td>23</td>
<td>6.0</td>
<td>189</td>
</tr>
</tbody>
</table>

Sporulation was measured after 45 hours in sporulation medium. For each strain, about 200 cells were scored for the production of asci containing two or more spores. Spore viability was assayed by dissection of ≥10 tetrads from each strain (only cells that have undergone both nuclear divisions form tetrads). The total DNA in heteroduplex is the maximum value obtained for each strain.

(e.g., 25 hr in Figure 3D vs. 20 hr in Figure 2A). This might reflect a genuine difference between wild type and mutant in the kinetics of hDNA formation. Alternatively, the apparent difference in timing could be due to the fact that sporulation is delayed in the zip1 mutant and consequently hDNA is not lost from DNA preparations until later time points.

Assay for homologous chromosome pairing: To assay homolog pairing, meiotic chromosomes were surface spread and painted with probes for chromosomes I and III. Only nuclei containing clear and compact hybridization signals for both chromosomes I and III were scored (see Loidl et al. 1994). Signal compaction reflects chromatin condensation, which reaches a maximum at pachytene (Dresser and Giroux 1988; Loidl et al. 1994). Chromosome I was detected as a red signal and chromosome III as a green signal. Two separate spots of the same color in one nucleus indicated that the homologs were unpaired. Chromosomes were classified as associated if they were so close together that their FISH signals had fused into a single spot or if the signals were aligned (i.e., touching each other).
were used to monitor homolog pairing. This strain has medium, when chromosome condensation and SC formation are maximal (ENGEBRECHT and ROEDER 1990). Cells were harvested at 15 hr after introduction into sporulation medium, when chromosome condensation is significantly lower than in wild type. The observed ability among experiments and the average level of pairing, and the extent of pairing is very reproducible from one experiment to another (Figure 4). Approximately 100 nuclei were scored in each experiment. Asterisks indicate condensation values that are significantly different from the wild-type value ($P < 0.05$).

The wild-type strain, BR2495, and isogenic derivatives were used to monitor homolog pairing. This strain has been used extensively in previous cytological studies and displays excellent preservation of meiotic prophase structures during spreading (e.g., ENGBRECHT and ROEDER 1990; ROCKMILL and ROEDER 1991). Cells were harvested at 15 hr after introduction into sporulation medium, when chromosome condensation and SC formation are maximal (ENGEBRECHT and ROEDER 1990). At this time point, 28% of the nuclei in wild type had condensed chromosomes (Table 4) and 95% of the chromosomes assayed were homologously paired (Figure 4).

**Homolog pairing in meiotic mutants:** The meiotic mutants tested can be divided into two categories, based on the results of the FISH assay of chromosome pairing. The mei4 and zip1 mutants display approximately wild-type levels of pairing, and the extent of pairing is very reproducible from one experiment to another (Figure 4). The remaining mutants display much greater variability among experiments and the average level of pairing is significantly lower than in wild type. The observed variability may reflect the instability of the associations between homologs in vivo and consequent susceptibility to the spreading procedure (see DISCUSSION). The average level of pairing observed in the mei4, rec102, hop1, red1, and mer1 mutants is ~30% of the wild-type value (Figure 4).

The FISH procedure also allows chromatin condensation to be assessed. The percent of nuclei that present clear and compact FISH signals represent the fraction of cells in which chromatin is condensed. Chromatin condensation in meiotic mutants was assessed at 15 hr after the introduction into sporulation medium, when the maximum number of wild-type cells were in pachytene. Previous studies have shown that all of the mutants examined progress to the pachytene stage of meiosis with normal kinetics in a BR2495 strain background (ENGEBRECHT and ROEDER 1990; ROCKMILL and ROEDER 1990, 1991; BHARGAVA et al. 1991; MENEES et al. 1992; SVM et al. 1993; B. ROCKMILL and G. S. ROEDER, unpublished results). Chromatin condensation was not different from wild type in the mei4, rec102 and mek1 mutants (Table 4). However, in the mer1, red1 and hop1 mutants, chromosome condensation was reduced significantly (Table 4). In zip1, the number of nuclei containing condensed chromosomes was twice the wild-type level (Table 4).

**DISCUSSION**

**MEI4 and REC102:** Previous studies have shown that mei4 and rec102 null mutants undergo absolutely no meiotically induced recombination, as assayed genetically (MENEES and ROEDER 1989; BHARGAVA et al. 1991; COOL and MALONE 1992). We have shown that the mei4 and rec102 mutations also prevent the formation of hDNA detectable in our assay (Figure 2B, Table 3), suggesting that these gene products act before strand exchange in the recombination process. This conclusion is consistent with previous studies demonstrating that the spore inviability of these mutants is alleviated in a spe13 background (MENEES and ROEDER 1989; BHARGAVA et al. 1991; MALONE et al. 1991; COOL and MALONE 1992). The spe13 mutation causes meiotic cells to undergo a single round of chromosome segregation to produce two-spored asc containing diploid spores (KLAPHOLZ and ESPOSITO 1980; HUGERAT and SIMCHEN

<table>
<thead>
<tr>
<th>Strain (relevant genotype)</th>
<th>Nuclei with condensed chromosomes (%)</th>
<th>Wild-type chromosome condensation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR2495 (wild type)</td>
<td>27.8*</td>
<td>100</td>
</tr>
<tr>
<td>SI574 (mei4)</td>
<td>20.4</td>
<td>73</td>
</tr>
<tr>
<td>JB278 (rec102)</td>
<td>24.0</td>
<td>86</td>
</tr>
<tr>
<td>BR2498 (hop1)</td>
<td>12.3*</td>
<td>44</td>
</tr>
<tr>
<td>BR2486 (red1)</td>
<td>16.9*</td>
<td>61</td>
</tr>
<tr>
<td>BR2496 (mer1)</td>
<td>15.7*</td>
<td>58</td>
</tr>
<tr>
<td>BR2626 (mek1)</td>
<td>28.3</td>
<td>102</td>
</tr>
<tr>
<td>MY65 (zip1)</td>
<td>54.9*</td>
<td>198</td>
</tr>
</tbody>
</table>

The percentages of nuclei with condensed chromosomes are the averages obtained in several different experiments for each strain. The number of experiments performed was six for wild type, six for mei4, six for rec102, five for hop1, nine for red1, six for mer1, five for mek1 and three for zip1. Approximately 100 nuclei were scored in each experiment. Asterisks indicate condensation values that are significantly different from the wild-type value ($P < 0.05$).
1993). Some sporulation-proficient, meiotic-lethal mutants produce viable spores in a spo13 background, whereas others produce dead spores. Genetic and physical analyses suggest that the former are defective in the initiation of meiotic recombination, whereas the latter initiate recombination, but fail to resolve recombination intermediates (Petes et al. 1991). Null mutations at the spo11, rad50, XRS2, MRE11 and MER2 loci (which are spo13-rescued) prevent the formation of meiosis-specific double-strand breaks (Cao et al. 1990; Ivanov et al. 1992; Jozuka and Ogawa 1995; Rockmill et al. 1995), the presumed initiators of most or all meiotic recombination events (Sun et al. 1989; Cao et al. 1990; Zenvirt et al. 1992).

Current models for meiotic recombination assume that hDNA is formed soon after the formation of double-strand breaks (Orr-Weaver and Szostak 1989). However, in temporal studies of wild-type yeast, hDNA was not detected until relatively late in meiotic prophase, at approximately the same time as crossover products (Goyon and Lichtten 1993; Nag and Petes 1993). (These studies employ mismatches that are poorly recognized by the mismatch repair machinery; consequently, any hDNA formed persists.) It is possible that hDNA is present earlier, but cannot be detected in the assays employed. For example, hDNA might be lost because of branch migration during DNA extraction and/or hDNA might be contained in branched or gapped molecules that fail to migrate as a single species during gel electrophoresis (Goyon and Lichtten 1993). Under these circumstances, hDNA would be detected only late in prophase, after the resolution of recombination intermediates. Thus, a failure to detect hDNA in meiotic mutants should be interpreted with caution. Mutants such as mei4 and rec102 might make heteroduplex that is not stabilized and therefore not detected in established assays.

Studies of spread chromosome preparations demonstrate that the mei4 and rec102 mutants assemble short stretches of axial elements, but no tripartite SC (Bhargava et al. 1991; Menees et al. 1992). FISH analysis indicates that these mutants undergo chromatid condensation (Table 4), but homolog pairing is substantially reduced (Figure 4). The observation that homologs are paired in yeast cells that have not yet entered meiosis has led to the proposal that pairing in vegetative and meiotic cells involves the same mechanism (Kleckner and Weiner 1993; Weiner and Kleckner 1994). Because pairing in premeiotic cells is unlikely to involve double-strand breaks or other lesions in the DNA, it has been suggested that the initial associations between homologs involve unstable paramenic joints between intact DNA duplexes (Kleckner and Weiner 1993; Weiner and Kleckner 1994). During meiosis, the associations between homologs are stabilized by chromosome synopsis and perhaps also by the formation of recombination intermediates. Stabilization of paramenic joints might be necessary to counteract the forces of meiotic chromatin condensation, which could otherwise disrupt homolog pairing (Kleckner and Weiner 1993; Weiner and Kleckner 1994). In the mei4 and rec102 mutants, chromatin condensation proceeds, but there is no recombination or SC formation. Thus, one interpretation of the mei4 and rec102 mutant phenotypes is that these mutants are proficient in homology searching, but unable to stabilize the associations between homologs, as proposed for the spo11 and rad50 mutants (Weiner and Kleckner 1994). An alternative possibility is that the Mei4 and Rec102 proteins are directly involved in homology searching and/or recognition, but there exists an alternative mechanism that operates in their absence and accounts for the pairing observed.

The homolog pairing observed in the mei4 and rec102 mutants cannot be attributed entirely to residual premeiotic pairing in unsporulated cells. Pairing was scored in nuclei in which individual chromosomes were evident when visualized with a DNA-specific stain; chromosomes never reach this degree of condensation in premeiotic cells. Furthermore, the level of pairing observed in the mei4 and rec102 mutants is higher than in the spo11 mutant (12%, based on the analysis of a spo11 derivative of BR2495 using procedures identical to those described here) (Rockmill et al. 1995). spo11 and all of the mutants analyzed in this study enter meiosis as efficiently as wild type (Klapheck et al. 1985; Engbrecht and Roeder 1990; Rockmill and Roeder 1990, 1991; Bhargava et al. 1991; Menees et al. 1992; Loidl et al. 1994; Sym and Roeder 1994). If all of the homolog pairing observed in spo11 is assumed to be residual premeiotic pairing, then the level of meiotically induced pairing in any given mutant is the difference between the spo11 pairing value and the corresponding mutant value. In the case of mei4 and rec102, this difference is 15–16%.

In the FISH assay, we observed considerable variability in the level of chromosome pairing for several mutants, including mei4 and rec102. We suggest that this variability is due to weakness of the associations between homologous chromosomes in vivo. Unstable joints may be very sensitive to minor variations in the preparation of cells and spreading of chromosomes. Thus, the pairing values obtained from spreads might reflect both the extent of pairing in vivo and the extent to which pairing is preserved during spreading. According to this view, the highest pairing value observed among spread preparations most closely approximates the pairing that occurs in intact cells.

**HOP1 and RED1:** Antibodies to the Hop1 and Red1 proteins localize to the SC and to unsynapsed axial elements (Hop1: Hollingsworth et al. 1990; F. Klein and B. Byers, personal communication; Red1: A. Smith and G. S. Roeder, unpublished results), raising the possibility that these proteins are components of axial and
lateral elements. Overproduction of the Red1 protein suppresses certain nonnull alleles of the *HOP1* gene, suggesting that these proteins physically interact with each other (HOLLINGSWORTH and JOHNSON 1993; FRIEDMAN et al. 1994).

Both red1 and hop1 null mutants display very low spore viability (1%) and this lethality is rescued by a spo13 mutation (ROCKMILL and ROEDER 1988; HOLLINGSWORTH and BYERS 1989). In red1 spo13 diploids, gene conversion occurs at 2–100% of the wild-type level, depending on the locus examined, and crossing over is reduced about fourfold (ROCKMILL and ROEDER 1990). In red1 strains, hDNA formation at HIS4 is 8% of wild type (Figure 3A, Table 3), in agreement with the observation that the level of gene conversion at HIS4 is 9% of wild type (ROCKMILL and ROEDER 1990). Intercellular recombination in the hop1 null mutant is only ~1% of the wild-type level (ROCKMILL and ROEDER 1990), consistent with the failure to detect hDNA (Figure 2C, Table 3). The hop1 mutant sustains ~10% of the wild-type level of double-strand breaks, but most or all of these lesions appear to participate in sister-sister exchanges, instead of recombination between nonsister chromatids (SCHWACHA and KLECKNER 1994). Strand exchange between sister chromatids would not generate hDNA and therefore would not be detected in our assay.

Analysis of silver-stained spread preparations of meiotic chromosomes reveals that red1 has the most severe cytological phenotype of any mutant characterized to date. The red1 null mutant fails to assemble any axial elements or tripartite SC (ROCKMILL and ROEDER 1990). Two observations led LOIDL et al. (1994) to propose that axial elements might impose a chromatin configuration that facilitates homology searching. First, all mutants previously shown to display meiotically induced chromosome pairing make at least short stretches of axial elements (ALANI et al. 1990; ENGBRECHT and ROEDER 1990; BHARGAVA et al. 1991; ROCKMILL and ROEDER 1991; MENNES et al. 1992; SYM et al. 1993; LOIDL et al. 1994). Second, *Schizosaccharomyces pombe* makes structures that strongly resemble axial elements even though this organism does not make any tripartite SC (BAHLER et al. 1993). The fact that the red1 mutant undergoes some meiotically induced homolog pairing, in the absence of axial elements, indicates that these structures are not absolutely required for homology searching and recognition.

The level of homolog pairing observed in the red1 mutant is the same as in mei4, rec102, and hop1 strains. Whereas the mei4, rec102 and hop1 mutants undergo little or no meiotically induced interchromosomal exchange, crossing over in the red1 null mutant is ~25% of wild type. If the formation of recombination intermediates stabilizes pairing interactions (WEINER and KLECKNER 1994), then the red1 mutant should show a higher degree of homolog pairing than mei4 and rec102 strains. Thus, these observations suggest that recombination is not sufficient to stabilize pairing, but they do not preclude the possibility that recombination events in the context of axial elements lead to stable associations between homologs. Also, the possibility should be considered that the level of pairing in vivo (i.e., before spreading) in red1 strains is higher than in mutants with more severe recombination defects.

In our experiments, the hop1 mutant showed a low level of homolog pairing (29%), similar to the mei4 and rec102 mutants (Figure 4). This result contrasts with the results of LOIDL et al. (1994) and WEINER and KLECKNER (1994) who reported pairing in hop1 at 55 and 87%, respectively, of the wild-type value. One obvious explanation for the difference between our results and those reported previously is yeast strain background. We measured pairing in a BR2495 strain, whereas other investigators have used the rapidly sporulating strain, SK-1. Perhaps unstable associations between homologs are particularly fragile in BR2495 and consequently more easily disrupted during spreading. Differences in spreading protocol may also be partly responsible. In addition, WEINER and KLECKNER (1994) used relatively short DNA probes, which allowed them to measure pairing in all nuclei, independent of the extent of chromatin condensation. If homologs pair in the hop1 mutant, but the associations between homologs tend to be disrupted during condensation, then this could account for the higher pairing values obtained by WEINER and KLECKNER (1994) as compared with our results and those of LOIDL et al. (1994). Finally, each of the pairing values presented by LOIDL et al. (1994) is the average of the three experiments with the highest pairing levels for each strain, whereas we have presented averages that include the results of all experiments. It should be emphasized that the strains we have analyzed by FISH are isogenic and all spreads have been prepared using the same protocol. Thus, the results obtained with different mutants are comparable.

The red1 and hop1 mutants both display defects in chromosome condensation (red1 and hop1, Table 4; hop1, LOIDL et al. 1994; WEINER and KLECKNER 1994). These results are not surprising because both of these proteins are associated with axial and lateral elements, which are thought to be related to mitotic chromosome scaffolds. The DNA in meiotic chromosomes is organized as a series of chromatin loops, each attached at its base to an axial or lateral element (MOENS and PEARLMAN 1988). An alteration in these chromosome cores might be expected to result in a defect in chromatin organization.

**MER1:** The MER1 gene encodes a meiosis-specific RNA splicing factor that is required for processing the transcript of *MER2* and probably at least one additional gene (ENGBRECHT et al. 1991; NANDABALAN et al. 1993; NANDABALAN and ROEDER 1995). In the absence of the
Mer1 protein, the *MER2* transcript is spliced at only 10% of the wild-type level. 

The *mer1* mutant null displays ~10% of the wild-type level of meiotic recombination and a substantial defect in chromosome synapsis. The *mer1* defect affects gene conversion and SC formation are consequences of the defect in *MER2* RNA splicing, because introduction of an intronless version of the *MER2* gene into a *mer1* mutant fully restores conversion and chromosome synapsis. The decrease in hDNA formation in *mer1* to 14.5% of the wild-type level (Figure 3C, Table 3) arises because of the fact that the spreading procedure partially disrupts this defect (ENGBRECHT and ROEDER 1989). The hDNA formation in *mer2* null mutant reflect the low level of *MER2* RNA splicing that occurs in the absence of Mer1 protein. Deletion of the *MER2* gene completely eliminates meiotic gene conversion (ENGBRECHT et al. 1991; COOL and MALONE 1992; ROCKMILL et al. 1995) and presumably hDNA formation.

In spread chromosome preparations from the *mer1* mutant, ~10% of the nuclei display fully synapsed chromosomes, whereas the remaining 90% exhibit full-length, but unsynapsed axial elements (ENGBRECHT and ROEDER 1990). The *mer2* mutant assembles axial elements of variable length, but no mature SC. Homolog pairing in a *mer2* strain is 28% (ROCKMILL et al. 1995); thus, the pairing level of 35% observed in the *mer1* mutant is roughly equivalent to the *mer2* value plus 10%. In contrast to our results, WEINER and KLECKNER (1994) reported homolog pairing in *mer1* at 77% of wild type. As suggested above for *hop1*, the higher pairing values observed by WEINER and KLECKNER (1994) might be due to their ability to monitor chromosome pairing at all stages of meiosis and/or to greater stability of the associations between homologs in an SK-1 strain background.

In spread chromosome preparations from the *mer1* mutant, most nuclei display full-length axial elements, but no SC. Despite the fact that the *mer1* mutant shows a significant level of homolog pairing, there is no obvious parallel alignment of axial elements of equivalent length in silver-stained preparations. The fact that axial elements are not obviously aligned implies that some or all interactions between homologs take place in chromatin loops distant from the cores of the chromosomes, as suggested previously for other meiotic mutants (LOIDL et al. 1994). In spread nuclei, wild-type SCs are surrounded by a diffuse mass of chromatin that is about 10 times the distance between lateral elements within an SC (1000 vs. 100 nm) and one-tenth to one-fifth the diameter of a spread nucleus (MOENS and PEARLMAN 1988). It is possible that some or all homologs are aligned along their entire length in intact *mer1* cells, but that the spreading procedure partially disrupts this pairing (and perhaps also increases the distance between paired axial elements).

**MEKI:** The *MEK1* gene encodes a meiosis-specific protein kinase homolog (ROCKMILL and ROEDER 1991; LEEM and OGAWA 1992) that localizes to the nucleus (BURNS et al. 1994). The spore viability of a *mek1* null mutant ranges from <1–13% depending on strain background; spore viability is improved by a *sto13* mutation (LEEM and OGAWA 1992; ROCKMILL and ROEDER 1991). Genetic analysis of *mek1* strains indicates that meiotic gene conversion and crossing over are decreased approximately 10-fold (ROCKMILL and ROEDER 1991; LEEM and OGAWA 1992). The *mek1* mutation reduces hDNA formation at 14% of wild type (ROCKMILL and ROEDER 1991). The *mek1* mutation also affects a 10-fold reduction in the steady-state levels of meiosis-specific double-strand breaks (LEEM and OGAWA 1992).

The *mek1* mutant undergoes extensive SC formation, but the stretches of SC assembled are shorter and more numerous than in wild type (ROCKMILL and ROEDER 1991). It was suggested that chromosome synapsis in *mek1* might involve nonhomologous chromosomes and chromosome segments (ROCKMILL and ROEDER 1991). There is extensive SC formation between nonhomologous chromosomes during meiosis in haploid yeast, but the stretches of SC formed are generally shorter than those observed in wild type (LOIDL et al. 1991). Our FISH analysis demonstrates that homolog pairing occurs at a wild-type level in the *mek1* mutant (Figure 4), indicating that the SCs observed in silver-stained preparations represent synapsis between homologs. *mek1* is the only mutant characterized to date that undergoes a nearly wild-type level of homologous chromosome synapsis, despite a substantial reduction in meiotic interchromosomal recombination.

**ZIP1:** The *ZIP1* gene encodes a structural component of the central region of the SC (SYM et al. 1993; SYM and ROEDER 1995). In the absence of the *Zip1* protein, axial elements of similar length line up side-by-side, but the distance between them is variable and greater than the distance between lateral elements in mature SC. Anti-Zip1 antibodies localize to the SC, but not to unsynapsed axial elements. The internal region of the Zip1 protein is predicted to form an α-helical coiled coil, and mutations that increase the length of the coiled coil increase the distance between lateral elements in tripartite SC. This observation suggests that the Zip1 protein lies perpendicular to the long axis of the complex and may be a component of the transverse filaments of the SC.

The *zip1* mutant sporulates in some yeast strain backgrounds, but not others, perhaps due to variation between strains in the operation of a meiotic cell cycle checkpoint (SYM et al. 1993; SYM and ROEDER 1994). In an SK-1 strain background, the *zip1* mutant sporulates and produces almost 60% viable spores, making it possible to examine the effect of *zip1* on meiotic recombin-
tion and chromosome segregation by tetrad analysis (SYM and ROEDER 1994). Although there is some variation from locus to locus, the zip1 mutation does not significantly affect the overall frequency of gene conversion. However, crossing over in the zip1 mutant is reduced two- to fourfold. Furthermore, the zip1 mutation completely eliminates crossover interference. As a consequence of the reduction in crossing over and the deregulation of crossover distribution, small chromosomes frequently fail to cross over and therefore nondisjoin.

The observation that zip1 undergoes wild-type levels of gene conversion suggests that recombination initiates normally. However, the decrease in crossing over implies that there is a decreased probability that recombination intermediates will be resolved in favor of crossing over. The zip1 mutation does not decrease hDNA formation (Figure 3D, Table 3), consistent with the notion that Zip1 acts late in the recombination pathway, perhaps affecting the resolution of Holliday junctions. There are three possible explanations for the observed increase in hDNA formation in the zip1 mutant. First, this might be an artifact of the delay in sporulation in the zip1 mutant, resulting in better recovery of hDNA at late time points, as discussed above. Second, in the DNY strain background, the zip1 mutant might be delayed at a stage in meiosis at which recombination events initiate, leading to higher than the wild-type level of recombination events. Third, the increase in hDNA might simply reflect locus-specific variations in the effect of the zip1 mutation on meiotic gene conversion.

In the BR2495 strain used to assay homolog pairing, the zip1 mutant displays a uniform arrest in meiotic prophase. Although previous studies indicated that zip1 arrests in diplotene or diakinesis (SYM et al. 1993); more recent work indicates that the zip1 mutant arrests in pachytene with axial elements intact and homologously paired (B. ROCKMILL, K.-S. TUNG and G. S. ROEDER, unpublished results). Arrest at pachytene when chromatin is maximally condensed can account for the increase in the percentage of nuclei with condensed chromatin as observed by FISH (Table 4).

The high level of chromosome pairing in the zip1 mutant as determined by FISH (Figure 4) is consistent with the observed parallel alignment of axial elements of equivalent length in silver-stained spread nuclei (SYM et al. 1993). In these preparations, it is evident that there are a few sites along each chromosome pair at which axial elements are connected to each other, as if synapsis had initiated, but the SC is unable to zipper up. The number of these connections corresponds approximately to the number of crossovers that occur in the zip1 mutant. This correspondence in number, together with the cytological similarities between the connections between chromosomes observed in zip1 spreads and the chiasmata seen at diplotene in higher organisms, led to the suggestion that the connections correspond to sites of crossing over (SYM et al. 1993; SYM and ROEDER 1994).

Tetrad analysis of zip1 SK-1 strains indicates that chromosome III fails to cross over in ≥20% of meioses (SYM and ROEDER 1994). This number is expected to be much higher for chromosome I, which is significantly smaller than chromosome III. Yet 97% of both chromosomes I and III are homologously paired in a zip1 BR2495 strain as evidenced by FISH. Thus, these data suggest that initiation of a crossover is not required to establish a stable association between homologs in the zip1 mutant. It is impossible to measure crossing over in the zip1 BR2495 strain used for FISH analysis because these cells fail to sporulate. However, at 15 hr of sporulation (i.e., when the FISH was carried out), commitment to crossing over (ESPONI and ESPONI 1974) in zip1 BR2495 is the same as in wild type (data not shown). In the absence of crossover interference, even a wild-type number of crossovers should not be sufficient to ensure that every chromosome pair recombines. Assuming that crossovers are distributed as a function of the physical sizes of chromosomes (consistent with studies of zip1 SK-1) (SYM and ROEDER 1994), ~20% of the chromosome I pairs and ~6% of the chromosome III pairs should fail to crossover. Nevertheless, the FISH analysis indicates that even chromosome I is paired in 97% of nuclei with condensed chromosomes. Thus, if the connections between axial elements observed cytologically are responsible for holding homologs together (as their appearance suggests), then these connections are probably not sites of crossing over.

Summary and overview: Our studies of hDNA formation in meiotic mutants has revealed a strong correlation between the level of hDNA and the frequency of meiotic gene conversion, as measured genetically. These results establish the hDNA assay as an excellent physical measure of the strand exchange reactions that lead to postmeiotic segregation and gene conversion. In no case did we detect hDNA in the absence of genetic recombination. This result is consistent with studies indicating that mismatch correction is very rapid in yeast (Goyon and LICHTEN 1993; HABER et al. 1993). Once hDNA is formed, any mismatched base pairs appear to be corrected almost immediately with the genetic consequence of gene conversion.

Our FISH analysis of meiotic chromosome pairing has shown that several mutants with defects in SC formation also display substantially reduced levels of homolog alignment. Homolog pairing occurred at approximately the same low level in the mer4, rec102, hop1, mer1 and red1 mutants, even though meiotic recombination in these mutants ranges from undetectable to ~25% of the wild-type level. Thus, these results provide no evidence that recombination (i.e., strand exchange) plays a role either in homology searching or in stabilizing the associations between homologous chromosomes. In this study, mutants with defects in chromosome pairing displayed substantial variation in pairing values from one experiment to another. We suggest that this vari-
ability reflects the instability of the associations between homologs in vivo and a consequent failure to preserve all such associations during spreading. To obtain an accurate measure of pairing in vivo, it may be necessary to assess homolog pairing in whole meiotic nuclei.

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