Meiotic Recombination Remote from Prominent DNA Break Sites in S. pombe

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Summary

DNA breakage is intimately associated with meiotic recombination in the fission yeast Schizosaccharomyces pombe. Sites of prominent DNA breakage were found ~25 to ~200 kb apart in the genomic regions surveyed. We examined in detail a 501 kb region of chromosome I and found six sites, or tight clusters of sites, at which ~2%–11% of the DNA accumulated breaks in a rad50S mutant. In contrast to the discrete, widely spaced distribution of prominent break sites, recombination in this region was more uniformly distributed (0.7–1.6 cM/10 kb) whether the genetic interval tested contained no, one, or more such sites. We infer that although recombination depends upon DNA breakage, recombination often occurs remote from these sites (tens of kilobases away); we discuss mechanisms by which this may occur.

Introduction

In most organisms, homologous genetic recombination occurs at high levels during meiosis to allow the pairing and subsequent reductional segregation of homologs and to enhance genetic diversity among the progeny (reviewed by Roeder, 1997, and Davis and Smith, 2001). In at least two species, the budding yeast Saccharomyces cerevisiae and the fission yeast Schizosaccharomyces pombe, meiotic recombination is initiated by double-strand (ds) DNA breakage (Sun et al., 1989; Cao et al., 1990; Cervantes et al., 2000). In some well-analyzed regions of the S. cerevisiae genome, DNA break sites occur roughly 5 kb apart (Baudat and Nicolas, 1997), and the broken DNA is frequently repaired by recombination with a homolog in a manner that produces recombinational exchanges within 1–2 kb of the broken ends (Lichten and Goldman, 1995; Smith, 2001). In contrast, in S. pombe meiotic DNA break sites appear to be farther apart, ~25 to ~200 kb, but recombinational exchanges are more uniformly distributed than these break sites (Cervantes et al., 2000; this report). These observations imply that recombination can frequently occur a substantial distance, ~100 kb or more, from the broken DNA ends.

Meiotic DNA breakage requires multiple gene products. In S. pombe, the Rec6, Rec7, Rec12, Rec14, and Rec15 proteins are essential for both meiotic recombination and DNA breakage (Cervantes et al., 2000; Davis and Smith, 2001). Thus, breakage and recombination are mechanistically linked in S. pombe, presumably by a precursor-product relation, as is the case in S. cerevisiae (Cao et al., 1990). A key component for DNA breakage in S. cerevisiae is the Spo11 protein, which becomes covalently linked to the 5’ ends of the broken DNA (Keeney et al., 1997). Tyr-135 of Spo11 is essential for DNA breakage and recombination; presumably Tyr-135 forms a phosphodiester link between Spo11 and the DNA by a topoisomerase-like mechanism (Bergerat et al., 1997; Keeney et al., 1997). The homologous Tyr-98 of the S. pombe homolog Rec12 is also essential for recombination, suggesting that the two proteins act by the same mechanism (Cervantes et al., 2000).

The repair of meiotic DNA breaks also requires multiple proteins (Roeder, 1997). In S. cerevisiae, the DNA 5’ ends are resected, and the resultant 3’ single-stranded (ss) tails invade a homolog to form a joint molecule. Resection requires the Rad50, Mre11, and Xrs2 proteins, and joint molecule formation requires Rad51 and Dmc1 proteins, homologs of the Escherichia coli RecA DNA strand exchange protein. Joint molecules, which include double Holliday junctions, are presumably resolved into recombinant molecules. Proteins responsible for resolution have not been identified in S. cerevisiae, but the Mus81-Eme1 protein complex of S. pombe appears to be essential for resolution of Holliday junctions (Boddy et al., 2001).

The Rad50 protein plays a dual role, being necessary for both the formation and the repair of DNA breaks. In S. cerevisiae rad50 deletion mutants, no DNA breakage is detected (Cao et al., 1990). In a special class of mis-sense mutants designated rad50S, breakage occurs but resection and subsequent steps of repair do not (Alani et al., 1990). The accumulation of broken DNA in rad50S mutants therefore reveals a more complete picture of the positions and extent of meiotic DNA breakage than that from wild-type cells. We used here an analogous mutant of S. pombe for this purpose. After determining the positions and extent of DNA breakage, we compared them with the distribution of recombination in a particular genomic region and found they differed markedly.

Results

Meiotic DNA Breaks Accumulate in an S. pombe rad50S Mutant

In S. pombe rad50S cells, meiotic DNA breaks appear shortly after premeiotic replication and persist for ~1 hr; the broken DNA is then repaired, presumably by homologous recombination with either a homolog or a sister chromatid (Cervantes et al., 2000). We compared the behavior of DNA from a rad50S mutant and found that, as in S. cerevisiae (Alani et al., 1990), DNA breakage occurred at the normal time, but the broken DNA was not repaired.

For this analysis, we used a rad50S mutant with an amino acid substitution K81I at the position corresponding to the K81I rad50S mutant of S. cerevisiae (Alani et al., 1990; Cao et al., 1990). The DNA breakage assay was performed as described before (Cao et al., 1990). A key component for DNA breakage in S. cerevisiae is the Spo11 protein, which becomes covalently linked to the 5’ ends of the broken DNA (Keeney et al., 1997). Tyr-135 of Spo11 is essential for DNA breakage and recombination; presumably Tyr-135 forms a phosphodiester link between Spo11 and the DNA by a topoisomerase-like mechanism (Bergerat et al., 1997; Keeney et al., 1997). The homologous Tyr-98 of the S. pombe homolog Rec12 is also essential for recombination, suggesting that the two proteins act by the same mechanism (Cervantes et al., 2000).

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al., 1990; Manolis et al., 2001; E. Hartsuiker, personal communication). To achieve high synchrony among the meiotically induced cells, pat1-114 (Ts) mutant cells were arrested in the G1 phase of the cell cycle by starvation for nitrogen; restoration of nitrogen and elevation of the temperature inactivates the Pat1-114 protein kinase and rapidly induces synchronous meiosis (Iino and Yamamoto, 1985; Nurse, 1985). After induction, DNA replication, assayed by flow cytometry, occurred at ~2 hr in both the rad50+ and rad50S cells (see supplemental data at http://www.molecule.org/cgi/content/full/9/2/253/DC1). DNA breakage, assayed by pulsed-field gel electrophoresis to separate the three S. pombe chromosomes and staining with ethidium bromide as described in Experimental Procedures. (A), haploid rad50+ strain GP353; (B), haploid rad50S strain GP2956; (C), diploid rad50+ strain GP338; (D), diploid rad50S strain GP3087. The bands in the mitotic (0 hr) lanes are, from top to bottom, the wells into which the DNA was loaded (W), chromosome I (5.7 Mb), chromosome II (4.6 Mb), and chromosome III (3.5 Mb). The smear (asterisk) is broken DNA that appears transiently in the rad50+ strains and accumulates in the rad50S mutants.

As in previous studies (Li and Smith, 1997; Cervantes et al., 2000), haploid and diploid pat1-114 (Ts) mutants behaved similarly. Inactivation of the Pat1-114 protein kinase induced the early events of meiosis, including DNA replication, breakage, and repair, with similar kinetics in both cell types (cf. Figures 1A and 1B). In the rad50+ cells, the chromosomes had returned to their intact state by 5 hr, but in the rad50S cells broken DNA persisted, and no intact DNA was detectable even at 6 hr (Figures 1A and 1B) or as late as 24 hr (data not shown).

In additional DNA remained broken in both haploid and diploid rad50S pat1-114 (Ts) mutants (Figures 1B and 1D). As shown later (see Figures 3 and 4), DNA breaks at particular sites also accumulated equivalently in haploid and diploid cells.

Thus, the S. pombe rad50S mutant appears, by this analysis, to be similar to the S. cerevisiae rad50S mutant; it is blocked in the repair of meiotic DNA breaks.

Figure 1. Meiotic DNA Breakage but not Repair in a rad50S Mutant Cells were harvested at the indicated times (hr) after induction of meiosis, and the DNA was analyzed by pulsed-field gel electrophoresis and staining with ethidium bromide as described in Experimental Procedures. (A), haploid rad50+ strain GP353; (B), haploid rad50S strain GP2956; (C), diploid rad50+ strain GP338; (D), diploid rad50S strain GP3087. The bands in the mitotic (0 hr) lanes are, from top to bottom, the wells into which the DNA was loaded (W), chromosome I (5.7 Mb), chromosome II (4.6 Mb), and chromosome III (3.5 Mb). The smear (asterisk) is broken DNA that appears transiently in the rad50+ strains and accumulates in the rad50S mutants.

Prominent Meiotic DNA Break Sites Are Far Apart

When specific chromosomal regions are examined by Southern blot hybridization, prominent but transient bands are seen with DNA from rad50+ cells; these bands indicate prominent sites of frequent cleavage located ~100 to ~300 kb apart in several regions of the genome (Cervantes et al., 2000). To determine if the same pattern occurs in rad50S cells, we surveyed three regions of the genome for the accumulation of broken DNA ends in rad50S cells. The first region surveyed was that near ura1 on chromosome I, near which a prominent break site was previously found in rad50+ cells (Cervantes et al., 2000). After pulsed-field gel electrophoresis, broken DNA from meiotically induced cells was analyzed by Southern blot hybridization using a radioactive probe from the ura1 gene, located about 0.75 Mb from the left end of chromosome I.

We interpret the band marked with an asterisk and those above it in Figure 2A as a reflection of DNA molecules extending from the left telomere through the ura1 probe sequence to sites of prominent breakage. The pattern of break sites or clusters of sites is similar in rad50S cells (Figure 2A) and in rad50+ cells (Cervantes et al., 2000). The prominent band marked with an asterisk in Figure 2A represents a frequently cleaved site or cluster of sites just to the right (the telomere-distal side) of the ura1 gene. This site, designated mbs1 (meiotic break site 1), is analyzed more thoroughly below. Other prominent break sites are located farther to the right of mbs1, as deduced from the more slowly migrating DNA fragments. Fragments migrating more rapidly than (below) that marked with the asterisk in Figure 2A must represent molecules with two meiotic breaks, since they are too short to extend from the telomere to the ura1 probe. The shortest of these fragments was ~300 kb long, suggesting that a second meiotic break rarely occurs within this distance to the left of a break at mbs1.

Other regions of the genome also have widely spaced sites at which meiotic breaks accumulated in the rad50S mutant. A radioactive probe from cosmid c869, located about 100 kb from the right end of chromosome I, revealed eight prominent bands, reflecting eight prominent break sites or clusters of sites located ~165 to ~1200 kb from the telomere (Figure 2B). The distances between these sites ranged from ~70 to ~250 kb.

We analyzed breaks on chromosome III after digesting the DNA with the rarely cutting enzyme SfiI, since the ends of this chromosome have heterogeneous lengths of rDNA (Fan et al., 1991) which would smear the bands from undigested DNA. One SfiI site is 78.9 kb from the beginning of the rDNA at the left end of chromosome III. A probe located immediately to the left (telomere proximal) side of this SfiI site revealed three prominent bands reflecting break sites at ~35, ~60, and ~80 kb from the SfiI site (Figure 2C). The repeating pattern of less prominent breakage, visible above the prominent bands in Figure 2C, presumably reflects meiotic break sites within each of the 10.9 kb rDNA repeats. A probe immediately to the right of the SfiI site revealed seven prominent break sites before the next SfiI site located...
384 kb away (Figure 2D). The distances between these break sites ranged from \(~25\) to \(~90\) kb.

Because of the very prominent meiotic break site \textit{mbs1}, near \textit{ura1}, first observed with \textit{rad50}^− cells (Cervantes et al., 2000), we have examined in detail the region surrounding \textit{mbs1}. Since \textit{ura1} is near the middle of the 501 kb NotI fragment J (Fan et al., 1989), we analyzed NotI-digested DNA using probes from the left and right ends of this NotI fragment. The left-end probe revealed a strong band reflecting frequent breakage at \textit{mbs1}, located \(~240\) kb from the left end of the NotI fragment (Figure 3A). Somewhat weaker bands were seen above and below the \textit{mbs1} band, reflecting break sites at approximately \(135\) (\textit{mbs2}), 330, 350, 420, and \(460\) kb from the left end of the NotI fragment. The right-end probe revealed meiosis-specific fragments of lengths complementary to those revealed by the left-end probe (Figure 3B). From the two sets of data and others not shown we estimated the positions of these prominent break sites (see below and Figure 6); the distances between these sites ranged from \(~20\) to \(~100\) kb.

Similar patterns of meiosis-specific breakage were observed with diploid cells. For example, the positions and extent of DNA breakage on the NotI fragment J, revealed by left- and right-end probes, were similar with diploid \textit{pat1-114} cells (Figures 3C and 3D) and with haploid \textit{pat1-114} cells (Figures 3A and 3B). DNA breakage in other regions of the genome also was similar in haploids and diploids (data not shown).

To determine if meiosis-specific breakage at these prominent sites depends on recombination functions, as does breakage of whole chromosomes (Cervantes et al., 2000), we examined DNA from a \textit{rad50S rec12} double mutant. In this strain, whole chromosomes remained intact up to \(6\) hr after meiotic induction (data not shown), and no detectable meiosis-specific breakage was seen on the NotI fragment J (Figures 3E and 3F). DNA breakage at each of these prominent sites and others examined in the genome depends upon multiple \textit{rec} gene products (Cervantes et al., 2000; data not shown) and therefore is intimately associated with meiotic recombination.

**Quantitation of Meiotic DNA Breakage**

To determine the fraction of DNA broken at the prominent sites on the NotI fragment J, we analyzed the Southern blot hybridizations shown in Figure 3 with a PhosphorImager. Figure 4 shows graphically the amount of hybridization with the left-end probe to NotI-digested DNA from \textit{rad50S rec}^− and \textit{rad50S rec12} mutant cells.
at 1 hr and 5 hr after induction of meiosis (Figures 3A, 3C, and 3E). Six prominent peaks were seen with rec− DNA at 5 hr after induction. Between these peaks, the amount of hybridization was near that observed with rec− DNA at 1 hr (before DNA replication) or with rec12 mutant DNA at 1 hr or at 5 hr. Hybridization to the latter three samples was approximately uniform throughout the lanes of the gel; no obvious meiosis-specific peaks were seen. The intensity of this background was approximately 1% of the intensity of the full-length (unbroken) NotI fragment J. Since this background is independent of meiotic induction and rec12 gene function, we presume it reflects mechanical breakage due to manipulation of the cells and DNA or to enzymatic breakage unrelated to meiotic recombination. We have therefore subtracted this background in the following analysis.

We estimate the fraction of meiosis-specific DNA breakage (5 hr minus 1 hr values) at each of the prominent sites on NotI fragment J to be, from left to right, 3.3 ± 0.5 (mbs2), 10.8 ± 0.3 (mbs1), 1.6 ± 0.4, 2.1 ± 0.2, 3.3 ± 0.4, and 4.6 ± 0.4% of the total DNA (intact NotI fragment plus discretely broken fragments). These estimates are the mean values ± the standard error of the mean of five independent inductions of the rad50S haploid strain GP2956 and one of the rad50S diploid strain GP3087, each probed from both ends of the NotI fragment. The total fraction of the NotI fragment J broken at these sites during meiosis was ~26%.

The amount of meiosis-specific DNA breakage between the prominent break sites is difficult to quantitate accurately because it is so close to the background level. Nevertheless, we estimate from the hybridizations analyzed above that the amount of meiosis-specific breakage in an interval between mbs1 and mbs2 (res2-ura1; see Figures 3 and 6) was 0.52 ± 0.56% in the rec− strains and 0.2% in the rec12::kan strain GP3135. Thus, ~0.3% of the DNA in the res2-ura1 interval was broken in a meiosis-specific, rec12-dependent manner. There was also very little breakage in the lys3-ura1 interval, to the left of mbs2. The six probing from the right side of the NotI fragment yielded 0.47 ± 0.27%, and those from the left yielded ~3.3 ± 1.8%. The latter data have more scatter, owing to a higher background of meiosis- and rec12-independent broken fragments in the size range of these measurements (Figures 3 and 4). These data indicate that the amount of meiosis-specific break-
Quantitation of Meiotic DNA Break Frequency

The Southern blots of rad50S DNA in Figures 3A, 3C, and 3E were analyzed with a PhosphorImager. A background level of radioactivity from the lightest part of each lane was subtracted from each value, and the total remaining level of radioactivity for each lane was normalized to the highest value. The 1 hr samples (thin lines) are before DNA replication and breakage, and the 5 hr samples after (thick lines; see supplemental data at http://www.molecule.org/cgi/content/full/9/2/253/DC1 and Figures 1–3). Red lines, haploid rec/H11001 strain GP2956; blue lines, diploid rec/H11001 strain GP3087; black lines, haploid rec12-170 strain GP3135. Electrophoresis of the DNA from the diploid rec/H11001 strain was slightly longer than that for the other strains. Before normalization, peak counts in the unbroken 501 kb band ranged from 36,700 to 60,200. Note that significant DNA breakage is seen only with the rec12 strains after meiotic replication.

Mapping a Prominent Meiotic Break Site mbs1

To examine the relation between the positions and extents of meiotic DNA breakage and of meiotic recombination, we determined more precisely the location of mbs1, near ura1. The mobility, relative to size markers, of the broken fragment designated mbs1 in Figures 3 and 4 and the nucleotide sequence of the S. pombe genome (Wood et al., 2002) indicated that mbs1 is located on a 64.4 kb Pmel fragment. Consequently, Pmel-digested DNA from a rad50S strain was electrophoresed and analyzed with probes from each end of this fragment. A single prominent meiosis-specific band was observed with each probe (Figure 5); their mobilities indicated that mbs1 is located 38 kb from the left end of this Pmel fragment. A 1.0 kb probe extending from 37.5 kb to 38.5 kb from the left end of this Pmel fragment revealed both the left and the right meiosis-specific fragments (data not shown), suggesting that a substantial fraction of the breakage occurs within or near this 1.0 kb interval, which we designate mbs1.

We quantitated the fraction of DNA from a rad50S strain broken at mbs1 or in the flanking regions of the 64.4 kb Pmel fragment (Figure 5). In this experiment, ~8% of the DNA was broken at mbs1 at 4.5 hr after meiotic induction. Outside mbs1, the extent of breakage at 4.5 hr was nearly the same as that at 0 hr, before meiotic induction. This analysis indicated that there was little if any meiosis-specific DNA breakage in this 64.4 kb interval other than at mbs1.

Frequency of Meiotic Recombination Surrounding mbs1

Having located a prominent meiotic DNA break site mbs1, we asked whether this site is a hot spot of meiotic recombination. To answer this, we compared the intensity of recombination in a genetic interval containing mbs1 with the intensity in other intervals, including those undergoing little or no breakage (Table 1 and Figure 6). The intervals tested collectively spanned 476 kb, or nearly all of the 501 kb NotI fragment analyzed for DNA breakage. One set of eight genetic markers consisted of single base pair transition mutations in nonessential genes with readily scored phenotypes (see supplemental data at the above URL); although they may alter chromosomal features such as chromatin structure, crosses employing these markers gave results similar to those employing only single base pair transitions.

Recombinant frequencies were determined by random spore analysis. Most of the recombinants detected likely arose by crossing over (reciprocal recombination),
since the lowest frequency of recombination measured was 2.6% and the frequency of gene conversion (nonreciprocal recombination) of most *S. pombe* markers is ~0.07% per spore (range for 31 markers is ~0.03% to ~0.4%; P. Munz, personal communication). Crosses were repeated, in some cases with the markers both in coupling and in repulsion, and similar results were obtained. A total of 30,341 marker-pair segregations from 78 crosses was analyzed. The frequency of recombination between two markers was converted to genetic distance (centimorgans or cM) using the mapping function of Haldane (1919), which assumes no crossover interference as is the case for *S. pombe* (Munz, 1994). The positions of the markers on the nucleotide sequence of the NotI fragment J (see supplemental data at http://www.molecule.org/cgi/content/full/9/2/253/DC1) allowed calculation of the intensity of recombination, cM/10 kb.

The results of this extensive analysis (Table 1 and Figure 6) indicate that the intensity of recombination among intervals with or without a prominent meiotic DNA break site varied less than 40% from the overall mean value. In particular, the intensity of recombination in the interval with the most prominent break site *mbs1* (10.8% breakage), *ura1–rqh1*, was 1.2 cM/10 kb, close to the mean (1.1) of all the intervals measured with single base pair mutations (Table 1A). The adjacent interval on the left, *res2–ura1*, had 0.72 cM/10 kb and ~0.5% breakage. The adjacent interval on the right, *rqh1–mis6*, had 1.3 cM/10 kb and 7.0% breakage. The interval with the highest intensity of recombination (*lys3–aur1, 1.6 cM/10 kb*) had little or no meiosis-specific breakage. Thus, these data indicate that the prominent meiotic DNA break sites are not sites of especially intense recombination, or hot spots of recombination.

The data from crosses with large insertion or substitution mutations, denoted with an asterisk in Table 1B and Figure 6, gave a similar picture. For example, the interval *res2–rqh1*, spanning *mbs1*, had 0.9 cM/10 kb, close to the mean of 0.92 cM/10 kb for this set of data. Two intervals with little or no meiotic DNA breakage, *pom1*~mkh1~ and *rad15–res2*, had 1.3 cM/10 kb, nearly the same as 1.2 cM/10 kb from the measurement of *rad15–res2* with single base pair transition mutations. Thus,
these data, like those in the previous set of crosses, fail to show a simple correlation between the site of meiotic DNA breakage and the site of meiotic recombination. Meiotic recombination in this region of the genome is dependent upon the rec12 and other rec genes. In crosses homozygous for rec12-117, among 470 viable spores tested none were recombinant in the lys3-37-ura1-61 interval, and among 582 only one was recombinant in the ura1-61-pro1-1 interval. Comparison with the rec+ data in Table 1 shows that the rec12 mutation reduced recombination in these intervals by a factor of \( \geq 50 \). Meiotic recombination in the lys3-ura1-pro1 region is reduced by a factor of \( \geq 25 \) also by a rec6, 7, 14, 15, or 16 mutation (DeVeaux and Smith, 1994; Lin and Smith, 1995; Evans et al., 1997; Ding and Smith, 1998). Thus, both meiotic recombination and meiotic DNA breakage in this region are dependent, as far as tested, upon the same set of rec gene products.

**Discussion**

We report here the occurrence of widely separated sites in the *S. pombe* genome at which meiosis-specific ds DNA breaks frequently arise; between these sites little or no breakage was detected. In contrast to the discrete distribution of these prominent DNA break sites, meiotic recombination was more uniformly distributed. Our results demonstrate that hot spots of meiotic DNA breakage are not hot spots of meiotic crossing over and that meiotic recombination occurs tens of kilobases from these prominent break sites. While we cannot completely rule out additional, low-level breaks, the available evidence discussed below indicates that the ds DNA breakage between the prominent sites is too infrequent to account for the amount of recombination in those intervals. We infer that in *S. pombe* meiotic recombination frequently occurs remote from broken DNA ends. We discuss below how this may occur.

The regions of the *S. pombe* genome surveyed here for meiotic DNA breakage and recombination appear representative of the genome. Widely spaced, prominent DNA break sites have been found in the six regions of the genome surveyed (Cervantes et al., 2000; Figures 2 and 3; data not shown). Meiotic recombination in *S. pombe* appears to be distributed uniformly throughout the genome, with an intensity of \( \approx 2 \) cM/10 kb; this range includes the mean intensity of 1.1 cM/10 kb for the intervals in the extensively studied 501 kb NotI fragment J (Table 1 and Figure 6). The *S. pombe* genome encompasses \( \approx 2200 \) cM and \( \approx 14 \) Mb, or 1.6 cM/10 kb on average (Munz et al., 1989; Wood et al., 2002). For 20 intervals spanning most of chromosome I, the intensities range from \( \approx 0.7 \) to \( \approx 3.4 \) cM/10 kb (calculated from

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**Table 1. Recombination Intensity in Intervals with and without Prominent DNA Break Sites**

<table>
<thead>
<tr>
<th>Interval</th>
<th>Crosses1</th>
<th>Tested1</th>
<th>R11</th>
<th>R22</th>
<th>% Recomb.</th>
<th>cM1</th>
<th>kb</th>
<th>cM/10 kb</th>
<th>Range</th>
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<tbody>
<tr>
<td>lys3-37–ura1</td>
<td>4</td>
<td>1033</td>
<td>52 52</td>
<td>10.1</td>
<td>11.2</td>
<td>79.0</td>
<td>1.42</td>
<td>1.05–1.85</td>
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</tr>
<tr>
<td>pom1*-mkh1*</td>
<td>4</td>
<td>1013</td>
<td>57 53</td>
<td>10.9</td>
<td>12.3</td>
<td>190</td>
<td>0.96</td>
<td>0.84–1.05</td>
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<td>res2 (J3)-ura1</td>
<td>6</td>
<td>2965</td>
<td>44 33</td>
<td>2.6</td>
<td>2.7</td>
<td>56.6</td>
<td>1.21</td>
<td>0.94–1.66</td>
<td></td>
</tr>
<tr>
<td>rad15-ura1</td>
<td>5</td>
<td>616</td>
<td>106 93</td>
<td>5.6</td>
<td>5.9</td>
<td>78.7</td>
<td>0.75</td>
<td>0.61–0.89</td>
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<tr>
<td>res2 (G1)-ura1</td>
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<td>60 55</td>
<td>9.7</td>
<td>10.8</td>
<td>90.8</td>
<td>1.19</td>
<td>1.07–1.33</td>
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<tr>
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<td>1510</td>
<td>131 146</td>
<td>18.3</td>
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<td>198</td>
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<tr>
<td>ura1-61–pro1-1</td>
<td>6</td>
<td>4068</td>
<td>86 81</td>
<td>4.1</td>
<td>4.3</td>
<td>56.6</td>
<td>1.21</td>
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<tr>
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<td>77 92</td>
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<td>26.4</td>
<td>107</td>
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<tr>
<td>ura1-mis6</td>
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<td>60 55</td>
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<td>90.8</td>
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<td>ura1-rqh1</td>
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<td>56.6</td>
<td>1.21</td>
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<td>17 19</td>
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<td>66.1</td>
<td>0.95</td>
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</table>

1 | The total number of random spores analyzed from the number of crosses indicated. Data from individual crosses were similar and have been pooled.
2 | R1 and R2 are the number of reciprocal recombinant types for each cross.
3 | The percent of recombinants was converted to centimorgans (cM) by Haldane’s formula cM = \( 50 \ln(1 - 2R) \), where R is the recombinant frequency.
4 | The number of recombinants (R1 + R2) in previously published crosses in which the reciprocal types were tallied together (Lin and Smith, 1995; Evans et al., 1997; Ding and Smith, 1998).
5 | Indicated by an asterisk (see supplemental data at http://www.molecule.org/cgi/content/full/9/2/253/DC1 for descriptions of the mutations).
recombination data provided by P. Munz and physical data for the S. pombe genome); the mean and median values are 1.7 and 1.2 cM/10 kb, respectively, similar to those for the NotI fragment J. We anticipate that the conclusions we draw from the data reported here will apply to other regions of the S. pombe genome.

Nature of Prominent Meiotic DNA Break Sites
We mapped one meiotic break site, mbs1, to an interval of a few kilobases (Figure 5). This interval is within a region of 7.2 kb between two lengthy coding sequences, SPAC4G8.03c and SPAC4G8.04, between which there is no extensive coding sequence. This 7.2 kb intergenic region is considerably larger than the average intergenic region of ~0.9 kb in S. pombe (Wood et al., 2002). Mapping additional meiotic break sites should show if they are typically located in large intergenic regions.

The paucity of prominent meiotic break sites in S. pombe may stem from the consequences of the breakage and its repair. Hot spots of gene conversion in S. pombe and S. cerevisiae are recipients of genetic information (Gutz, 1971; Lichten and Goldman, 1995): an active hot spot is lost when a chromosome with an active hot spot undergoes gene conversion with a chromosome lacking that hot spot. Thus, such hot spots are expected to be lost from natural populations. If, as in S. cerevisiae (Lichten and Goldman, 1995; Petes, 2001), S. pombe meiotic DNA break sites are hot spots of gene conversion, most break sites may have been converted away during evolution of S. pombe, perhaps more extensively within coding sequences than between them.

Relation between Meiotic DNA Breaks and Recombination
Both meiotic DNA breakage and meiotic recombination depend on multiple rec gene products—rec6, 7, 8, 10, 11, 12, 14, and 15 (Figure 3; Cervantes et al., 2000; Davis and Smith, 2001; data not shown). This dependence indicates that the two processes are mechanistically connected, presumably in a precursor-product relation as in S. cerevisiae (Cao et al., 1990). The accumulation of meiotic DNA breaks in the S. pombe rad50 mutant (Figures 1–3), as in the corresponding mutant in S. cerevisiae (Alani et al., 1990), supports this view. The considerable reduction, perhaps abolition, of meiotic recombination in five mutants, including rec12, suggests that most, perhaps all, meiotic recombination in S. pombe depends upon DNA breakage.

The DNA broken during meiosis must be repaired to form intact chromosomes and to produce viable meiotic products. Repair could occur by the rejoining of DNA ends or by interaction with a sister chromatid or a homolog. Only interaction with a homolog can give an observed genetic recombinant. Most models of recombination initiated by ds DNA breaks predict that one reciprocal exchange, which produces two crossover chromatids, requires exactly one ds break. Our observation of ~26% meiotic DNA breakage and ~50 cM in the lys3–pro1 interval (Table 1; Figure 4; and Results) suggests, within the context of these models, that nearly all of the ds DNA breaks are repaired as crossovers. Although our data appear accurate within a factor of two, indicating that many of the breaks are repaired as crossovers, we cannot exclude that some are repaired in other ways, including gene conversion without crossing over. If end joining or sister chromatid interactions occur, they would exacerbate the problem of accounting for the observed amount of crossovers by the observed amount of DNA breakage: such repair without genetic recombination would leave even fewer breaks to generate the observed amount of recombinants. The available data thus suggest that many and perhaps most or all of the ds DNA breaks result in a crossover.

The distributions of ds DNA breakage and of recombination do not, however, appear to be congruent: recombination appears much more uniformly distributed than DNA breakage. For example, the 91 kb ura1–rqh1 interval has 11 cM, or 1.2 cM/10 kb, and 11% DNA breakage at mbs1 (Table 1; Figures 4 and 6). In contrast, the 57 kb res2–ura1 interval has 4.1 cM, or 0.72 cM/10 kb, but only ~0.3% meiosis-specific, rec12–dependent DNA breakage. Thus, comparing the ura1–rqh1 and res2–ura1 intervals, DNA breakage is less frequent in the latter interval by a factor of ~20, but recombination is less frequent by a factor of only 2.6 and the recombination intensity (cM/kb) by a factor of only ~1.7. Similar discrepancies in the frequency of DNA breakage and frequency of recombination are seen in other intervals such as lys3–aur1 (11 cM with little or no DNA breakage in 86 kb) versus ral1–mis6 (14 cM with three prominent break sites having a total of 7.0% DNA breakage in 107 kb) (Table 1; Figures 4 and 6). Prominent break sites are located ~20 kb to the left and ~20 kb to the right of the res2–ura1 interval (Figure 6). These observations indicate that recombination in this interval occurs ~20 kb and perhaps >50 kb from the nearer prominent
break site. As noted above, we cannot completely rule out undetected, low-level breaks between the prominent DNA break sites. But the data presented here argue that such breakage is insufficient to account for the high level of recombination in intervals, such as res2-ura1 and lys3-aun1, between prominent break sites.

More generally, if recombination in S. pombe were limited to 1-2 kb around the prominent DNA break sites, as frequently appears to be the case in S. cerevisiae (Lichten and Goldman, 1995), the genetic map of S. pombe would have clusters of genes (those between break sites) separated by spaces of recombination (those containing break sites). Genes are widely distributed, however, on the S. pombe genetic map (Munz et al., 1989), typified by the analysis of the 0.5 Mb region shown in Figure 6. These results imply that recombination occurs a substantial distance (up to ~100 kb) from prominent DNA break sites. We infer, therefore, that in S. pombe meiotic recombination frequently occurs far from broken DNA ends.

Models for Recombination Remote from DNA Ends
We entertain two general ideas for how recombination can occur far from a DNA end, yet be dependent upon DNA breakage. First, a DNA break could send a signal, such as relief of torsion, within a domain of the chromosome. Recombination might then be able to occur, by an unspecified mechanism, anywhere within the relaxed domain. We know of no precedent for this proposal, but it is conceptually similar to the proposal for the regulation of transcription within chromosomal domains. Second, an entity could move from a DNA end along the DNA to a distant point, at which it promotes recombination. We consider two models for such a moving entity.

In the first model, the moving entity is a protein or protein complex which binds to the ds DNA end, travels along the DNA, and initiates DNA strand exchange at a distance from the break (Figure 7A). This model is the same as that proposed for the RecBCD pathway of recombination in E. coli (Smith et al., 1984), which is supported by much evidence (Kowalczykowski et al., 1994; Myers and Stahl, 1994; Smith, 1994, 2001). We note that in this pathway a ds DNA break is not a hot spot of recombination (Lam et al., 1974); rather, it is an entry site for the RecBCD enzyme, which travels through the DNA and produces, as far as 30 kb or more from the entry site, a single-stranded DNA tail that undergoes strand exchange with a homolog. In S. pombe, a similar traveling recombination machine might promote recombination as far as ~100 kb from the entry site.

In the second model, the moving entity is a special DNA structure. This structure might be a double Holliday junction formed by the mechanism proposed by Szostak et al. (1983) for ds break repair (Figure 7B). Though formed at the site of the ds break, the junctions could move in tandem by branch migration to a distant site. In this case, the resolution of the junctions could produce a crossover, and perhaps gene conversion, at the distant site. The ds break site might be a hot spot of gene conversion but would not be a hot spot of crossing over, since after branch migration there is no connection between the DNA duplexes at the break site. Tandem branch migration of the dual junctions would leave no hybrid DNA, and consequently no gene conversion or crossing over, between the ds break site and the site of resolution.

Some events at the ARG4 locus of S. cerevisiae may reflect the mechanism in Figure 7B. In one study (Gilbertson and Stahl, 1996), ~10% of the non-Mendelian asci reflect heteroduplex DNA on one chromatid on each side of a prominent DNA break site without associated crossing over. These events have been interpreted as
the result of resolution of a double Holliday junction by topoisomerase "pullout," the separation of the joined duplexes in the vertical direction as drawn in Figure 7B (third panel from the bottom). Tandem branch migration in the horizontal direction would, however, have the same genetic consequences near the site of the ds DNA break. Distant crossing over could occur following horizontal branch migration but not following topoisomerase pullout.

Meiotic recombination may, in some cases, occur far from a ds DNA break in S. cerevisiae. On chromosome III of S. cerevisiae, there is an ~100 kb region with very little meiotic DNA breakage but a substantial amount of crossing over (Baudat and Nicolas, 1997). This region is, at least superficially, similar to the ~100 kb region between mbs1 and mbs2 of S. pombe (Figures 4 and 6). At the S. cerevisiae HIS4 locus, there is, in numerous mutants, a good linear relation between the frequency of ds breaks and the frequency of non-Mendelian segregation; this line extrapolates, however, to ~10% non-Mendelian segregation with 0% DNA breakage (Fan et al., 1995). The wild-type strain manifests ~30% non-Mendelian segregation. Thus, about one-third of the meiotic recombination at HIS4 appears to be independent of ds breaks at this locus; these events may stem from distant DNA breaks or from another source.

These observations raise the possibility that meiotic recombination differs in the two distantly related yeasts S. pombe and S. cerevisiae primarily in the ratio of events near and far from DNA breaks (Smith, 2001). Meiotic recombination may be mostly near DNA breaks in S. cerevisiae and far from them in S. pombe.

**Experimental Procedures**

**Strains**

The S. pombe strains used for analysis of meiotic DNA carried the h 

114 end-148 ade-6262 markers plus the following: GP2956 [rad50 (K11)], GPP587 [rad50 rad50 + lys4-95 his4-239 + diploid], GP3135 [nad50 rec1-170:3HA-His-kanMX4]. Strains GP338 and GP355 were described by Cervantes et al. (2000). The strains used for recombination frequency determinations contained one, two, or three mutations in the lys3-pro1 interval on chromosome I (see supplemental data at http://www.molecule.org/cgi/content/full/9/2/253/DC1 for a description of these mutations); some contained additional mutations in ade6, ura4, or leu1, on chromosomes III, II, or I, respectively. The complete genotypes and genealogies are available upon request.

**Induction of Meiosis and Analysis of DNA**

Strains were grown, induced for meiosis, and analyzed for DNA content by flow cytometry as described (Cervantes et al., 2000). For analysis of DNA breakage, ~6 × 10^6 cells (30 ml of culture) were washed in cold 50 mM EDTA (pH 8.0) and converted to spheroplasts, either in liquid as done previously (Cervantes et al., 2000; Figures 1C, 2B–2D, and 5) or after embedding the washed cells in agarose (Figures 1A, 1B, 1D, 2A, and 3); this latter procedure gives more uniform yields of DNA and reduces mechanical breakage of DNA. The washed cells were suspended in 300 μl of CPEs (400 mM EDTA [pH 8.0], 120 mM NaH2PO4, 40 mM citric acid, 1.2 M sorbitol, 5 mM Na2SO4, 5 mM DTT, 1 mg/ml lyticase [Sigma Catalog #L-4025], and 5 mg/ml lysing enzymes [Sigma catalog #L-1412; final pH 7.0]), 400 μl 2% low-melting-point agarose in 0.25 M EDTA (pH 8.0), 1.2 M sorbitol was added, and the mixture was poured into 0.1 ml plug molds. After ~10 min at 4°C, the plugs were ejected into 1.8 ml of CPEs and incubated at 37°C for 1 hr. The buffer was removed and replaced with 1.8 ml of NDS/PK (0.5 M EDTA [pH 8.0], 10 mM Tris-HCl [pH 7.5], 10 mM Na2SO4, 1% Na lauryl sarcosine, 1 mg/ml Proteinase K). After incubation overnight at 50°C, the buffer was replaced with SDS/PK (NDS/PK with 0.5% SDS in place of Na lauryl sarcosine and Na2SO4). After incubation overnight at 50°C, the plugs were soaked in 2 ml of TE (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) + 0.5 mM PMSF for ~1 hr at room temperature and then in 2–3 washes of TE. Plugs were stored in TE at 4°C. DNA in the plugs was digested with restriction enzymes as recommended by New England Biolabs and analyzed by pulsed-field gel electrophoresis followed by Southern blot hybridization as described previously (Cervantes et al., 2000). The probes are described in the supplemental data at http://www.molecule.org/cgi/content/full/9/2/253/DC1. Quantitation was done with a PhosphorImager (Molecular Dynamics) and Excel software (Microsoft). Sizes of DNA fragments were estimated from S. cerevisiae chromosomal DNA and phage λ DNA (multimers and restriction digestion products) visualized by staining with ethidium bromide.

**Mutations Used for Recombination Analysis**

Each previously undetermined mutation in the lys3-pro1 interval was sequenced by PCR amplification of DNA fragments from one wild-type and two mutant strains. The entire open reading frame of the gene was sequenced from these fragments and compared with the sequence in GenBank. In each case, the two mutant sequences were identical to the wild-type sequence and that in GenBank, except for one base pair as indicated in the supplemental data (http://www.molecule.org/cgi/content/full/9/2/253/DC1). For previously determined mutations (aur1-t1, nhl1-t2, and mis6-302), DNA from a single mutant strain was sequenced and found to contain the reported single base pair difference from the GenBank sequence.

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