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Conserved and Nonconserved Proteins for Meiotic DNA Breakage and Repair in Yeasts

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ABSTRACT

During meiosis DNA double-strand breaks initiate recombination in the distantly related budding and fission yeasts and perhaps in most eukaryotes. Repair of broken meiotic DNA is essential for formation of viable gametes. We report here distinct but overlapping sets of proteins in these yeasts required for formation and repair of double-strand breaks. Meiotic DNA breakage in *Schizosaccharomyces pombe* did not require Rad50 or Rad32, although the homologs Rad50 and Mre11 are required in *Saccharomyces cerevisiae*; these proteins are required for meiotic DNA break repair in both yeasts. DNA breakage required the *S. pombe* midmeiosis transcription factor Mei4, but the structurally unrelated midmeiosis transcription factor Ndt80 is not required for breakage in *S. cerevisiae*. Rhp51, Swi5, and Rad22 + Rti1 were required for full levels of DNA repair in *S. pombe*, as are the related *S. cerevisiae* proteins Rad51, Sae3, and Rad52. Dmc1 was not required for repair in *S. pombe*, but its homolog Dmc1 is required in the well-studied strain SK1 of *S. cerevisiae*. Additional proteins required in one yeast have no obvious homologs in the other yeast. The occurrence of conserved and nonconserved proteins indicates potential diversity in the mechanism of meiotic recombination and divergence of the machinery during the evolution of eukaryotes.

In most eukaryotes homologous recombination occurs at high levels during meiosis to aid the proper segregation of homologs at the first meiotic division and to increase genetic diversity among gametes, the products of meiosis. The two meiotic cell divisions reduce the diploid number of chromosomes in the precursor cells to the haploid number in the gametes. The general mechanism of reductional segregation of homologs is highly conserved: in most eukaryotes recombination between homologs provides a physical connection between them that imparts tension when the homologs are properly arranged to segregate to opposite poles of the cell (Nicklas 1997). In the absence of recombination, homologs frequently missegregate, resulting in aneuploid gametes; the subsequent zygotic progeny are frequently sick or dead, underscoring the importance of understanding meiotic recombination.

The general mechanism of meiotic recombination may also be highly conserved: double-strand (ds) breaks in DNA may initiate meiotic recombination in most or all eukaryotes, and repair of these breaks by interaction with a homolog can produce recombinants. Meiotic ds breaks associated with recombination were first observed in the budding yeast *Saccharomyces cerevisiae* (Sun *et al.* 1989; Cao *et al.* 1990). Such breaks have been observed more recently in a second organism, the distantly related fission yeast *Schizosaccharomyces pombe* (Cervantes *et al.* 2000). There are two lines of indirect evidence for meiotic ds breaks in other organisms. First, various eukaryotes encode homologs of the Spo11 protein, which is essential for meiotic DNA ds break formation in *S. cerevisiae* (Keeney *et al.* 1997). Where tested in other organisms, these proteins are essential for meiotic recombination or viable gamete formation (Keeney 2001), and in *S. pombe* the homolog, called Rec12, is also essential for meiotic DNA ds break formation (Cervantes *et al.* 2000). Second, in mice a modified histone, γ-H2AX, thought to associate with chromatin specifically near ds breaks, appears as foci on meiotic chromosomes at the time expected for recombination; these foci are Spo11 dependent (Rogakou *et al.* 1998; Mahadevaiah *et al.* 2001).

The Spo11 protein of *S. cerevisiae* makes meiotic DNA ds breaks by cleaving phosphodiester bonds at nearby positions on complementary strands and becomes covalently linked to the 5′ DNA ends via phosphotyrosine bonds (Keeney *et al.* 1997; Keeney 2001). The tyrosine thought to make this bond, Tyr-135, is within an amino acid sequence that is similar in Spo11 proteins from diverse organisms. The corresponding tyrosine of *S. pombe* Rec12, Tyr-98, is essential for meiotic recombination (Cervantes *et al.* 2000). These observations suggest that the chemistry of meiotic DNA ds break formation is highly conserved.

Other aspects of the mechanism of DNA ds break formation in meiosis appear to be less conserved. *S.*

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cerevisiae Spo11 does not make meiotic DNA breaks on its own: nearly a dozen other proteins are required for ds break formation (Keeney 2001). Some, but not all, of these other proteins have homologs in *S. pombe* and other organisms. Similarly, some proteins in addition to Rec12 that are required for meiotic ds break formation in *S. pombe* do not have obvious homologs in *S. cerevisiae* (Davis and Smith 2001; see discussion). These nonconserved, additional proteins may regulate the timing and positioning of ds break formation by the conserved Spo11-related proteins.

The repair of ds breaks is complex and not yet as well understood as their formation. In *S. cerevisiae* the 5′ ends at ds breaks are resected a few hundred nucleotides; the resultant 3′ single-strand (ss) tails invade a homolog, producing a joint molecule that contains hybrid DNA (Schwacha and Kleckner 1994, 1995; Hunter and Kleckner 2001). Resection requires the Rad50•Mre11•Xrs2 complex, which has exonuclease activity, but this activity digests in the 3′ → 5′ direction, as does the homologous protein Rad50•Mre11•Nbs1 from humans (Haber 1998). Presumably, a different nuclease, or a controlling factor, complexed with Rad50•Mre11•Xrs2 is responsible for meiotic resection in *S. cerevisiae*. Joint molecule formation requires the *S. cerevisiae* Rad51 and Dmc1 proteins, homologs of the *Escherichia coli* RecA strand-transfer protein (Schwacha and Kleckner 1994, 1995; Sung *et al.* 2000; Sugawara *et al.* 2003; Wolner *et al.* 2003). This step is aided by several additional proteins, including Rad52, in *S. cerevisiae*. Proteins required for the resolution of joint molecules into recombinant molecules have not been firmly established, but recent evidence implicates the Mus81•Eme1 complex in resolution of joint molecules containing Holliday junctions into crossover recombinants in *S. pombe* (Boddy *et al.* 2001; Gaillard *et al.* 2003; Osman *et al.* 2003; Smith *et al.* 2003).

To assess the generality of the mechanism of meiotic recombination, we investigated the protein requirements for formation and repair of DNA ds breaks during meiosis in *S. pombe* and compared the results with published data from *S. cerevisiae*. We find here that certain homologous proteins are required for breakage or repair in both yeasts; other proteins, however, are required in one yeast but not in the other. These results imply that, although the basic ds break-join mechanism occurs in both yeasts, certain aspects of this mechanism, such as its regulation, differ in these distantly related organisms.

MATERIALS AND METHODS

Strains and culture media: The *S. pombe* strains used for meiotic DNA analysis and their genotypes are listed in Table 1. Sources of alleles inactivating recombination genes are in Table 1, footnote *a*. The alleles in the strains listed or in the relevant parent were confirmed by nucleotide sequencing of appropriate DNA fragments generated by PCR. Genealogies are available upon request. Culture media are described by Gutz *et al.* (1974).

Induction of meiosis and analysis of DNA: Strains were grown, induced for meiosis, and analyzed for cellular DNA content and meiotic DNA breakage as described by Young *et al.* (2002). In brief, cells were grown at 25° in appropriately supplemented Edinburgh minimal medium 2 (EMM2), arrested in G_1 by starvation for nitrogen, and induced for meiosis by adding NH_4Cl and raising the temperature to 34° to inactivate the *pat1-114* (Ts) protein kinase. At the indicated times cells were harvested by centrifugation, suspended in agarose plugs, and lysed with enzymes. After successive treatments with proteinase K and, when indicated, *Not*I restriction enzyme, the DNA in the plugs was subjected to pulsed-field gel electrophoresis. *Not*I-digested DNA was analyzed by Southern blot hybridization using radioactive probes homologous to the ends of the *Not*I fragment J; results with the right end probe (data not shown) were similar to those with the left end probe shown in Figure 2. Each mutant was induced two to five times with similar results.

Viable spore yields and meiotic recombinant frequencies: Haploid *pat1*$^+$ strains were grown to saturation in yeast extract liquid medium supplemented with adenine (100 μg/ml). Cells were mated and allowed to undergo meiosis at the indicated temperature on SPA sporulation agar or EMM2 minimal agar supplemented with all required nutrients. Haploid *pat1-114* strains were grown and induced for meiosis as described above. The ascus-cell suspensions were harvested after 2–3 days (*pat1*$^+$ strains) or at 24 hr (*pat1-114* strains) and treated with glusulase and EtOH to kill vegetative cells as described by Gutz *et al.* (1974). Total spore yields were determined by plating on yeast extract agar (YEA) supplemented with adenine (100 μg/ml), and Ade$^+$ spore yields were determined by plating on YEA-rich agar supplemented with guanine (200 μg/ml), which inhibits uptake of adenine (Cummins and Mitchison 1967). Viable spore yields are expressed as the number of viable spores produced divided by the number of viable *pat1*$^+$ cells of the less numerous parent in the mating mixture or by the number of viable *pat1-114* cells induced. Ade$^+$ recombinant frequencies are expressed as the Ade$^+$ spore yield divided by the total viable spore yield.

RESULTS

We prepared DNA from synchronously induced meiotic cells and assayed it for breakage using two types of pulsed-field gel electrophoresis (Cervantes *et al.* 2000; Young *et al.* 2002; see materials and methods). First, we analyzed the DNA under conditions that separate the three intact chromosomal DNAs—5.7, 4.6, and 3.5 Mb for chromosomes I, II, and III, respectively. DNA from wild-type (*i.e.*, *rec*$^+$ *rad*$^+$, etc.) cells before and up to 2 hr after induction of meiosis was mostly intact (Figure 1A). Between ~3 and 4 hr after meiotic induction, *i.e.*, after meiotic DNA replication was complete (data not shown; Cervantes *et al.* 2000), intact chromosomal DNA largely disappeared, and more rapidly migrating (broken) DNA appeared. After ~4 hr, intact chromosomal DNA reappeared, ~1 hr before the first meiotic division (see, for example, Li and Smith 1997). Broken DNA does not appear in *rec12* or in seven other *rec* mutants that are deficient in meiotic recombination, indicating that this breakage is intimately associated

TABLE 1

***S. pombe* strains for meiotic DNA break determination**

Strain	Genotype[a]
GP535	*h⁻ pat1-114 ade6-M26 end1-458*
GP813	*h⁺ swi5-39 pat1-114 ade6-M26 end1-458*
GP2496	*h⁺ rad32::ura4⁺ pat1-114 ade6-M26 ura4-D18*
GP3269	*h⁻ rad22::ura4⁺ rti1:: kanMX4 pat1-114 ade6-M26 ura4-D18 end1-458*
GP3574	*h⁻ mus81::kanMX pat1-114 ade6-M26*
GP3581	*h⁺ rhp51::his3⁺ pat1-114 ade6-M26*
GP3607	*h⁻ dmc1::ura4⁺ pat1-114 ade6-M26 ura4-D18*
GP3650	*h⁻ smt-0 mei4::ura4⁺ pat1-114 ade6-M26 ura4-D18*
GP3652	*h⁻ smt-0 rad50::kanMX6 pat1-114 ade6-M26*
GP4233	*h⁻ smt-0 rhp51::his3⁺ dmc1::ura4⁺ pat1-114 ade6-M26 his3-D1 ura4-D18*

[a] Mutations other than commonly used auxotrophies and mating-type alleles are described in the following references: *pat1-114* (Iino and Yamamoto 1985), *end1-458* (Uemura and Yanagida 1984), *rad32::ura4⁺* (Tavassoli *et al.* 1995), *rad22::ura4⁺* (van den Bosch *et al.* 2001), *rti1::kanMX4* (also known as *rad22B::kanMX4*; van den Bosch *et al.* 2002), *mus81::kanMX* (Boddy *et al.* 2001), *rhp51::his3⁺* (Grishchuk and Kohli 2003), *dmc1::ura4⁺* (Fukushima *et al.* 2000), *mei4::ura4⁺* (Horie *et al.* 1998), *rad50::kanMX6* (Hartsuiker *et al.* 2001), *swi5-39* (Schmidt *et al.* 1987), and *h⁻ smt-0* (Styrkarsdottir *et al.* 1993).

with meiotic recombination (Cervantes *et al.* 2000; Young *et al.* 2002).

Second, we analyzed the DNA after digestion with the rare-cutting restriction enzyme *Not*I, followed by electrophoresis and Southern blot hybridization with a probe homologous to the end of the 501-kb fragment J of chromosome I (Fan *et al.* 1989). We reported previously that this fragment contains six *m*eiosis-specific *b*reak *s*ites (*mbs*; or clusters of sites), including the prominent sites *mbs1* and *mbs2* (Young *et al.* 2002). Breakage at one or another of these sites produces DNA fragments that migrate more rapidly than the intact fragment J. In accord with the analysis of DNA without restriction enzyme digestion, DNA broken at these sites appeared between ~2.5 and 5 hr after meiotic induction but not before or after (Figure 2A). DNA broken at these sites does not appear in *rec* mutants, showing that breakage at these specific sites, like general chromosomal DNA breakage, is recombination associated (Cervantes *et al.* 2000; Young *et al.* 2002). We used these two assays to analyze meiotic DNA breakage and repair in mutants suggested by previous studies to be altered in breakage, repair, or both.

***S. pombe* Rad50 and Rad32 proteins are required for meiotic DNA repair but not for meiotic DNA breakage:** The *S. cerevisiae* Rad50•Mre11•Xrs2 complex has a dual role in meiotic recombination: it is required for both DNA ds breakage and repair (Haber 1998). The *S. pombe* Rad50 and Rad32 proteins have ~35 and 45% amino acid sequence identity with *S. cerevisiae* Rad50 and Mre11, respectively (Tavassoli *et al.* 1995; Hartsuiker *et al.* 2001). *S. pombe rad50* and *rad32* mutants are defective in repair of mitotic DNA ds breaks and in formation of viable gametes (spores; Tavassoli *et al.* 1995; Hartsuiker *et al.* 2001; see below). We therefore assayed these mutants for meiotic DNA breakage and repair.

In both *rad50*Δ and *rad32*Δ mutants broken DNA began to appear ~3 hr after meiotic induction, was abundant by ~4 hr, and remained detectable for up to 8 hr (Figure 1, B and C). In some inductions the intact chromosomal DNA did not disappear to the extent seen in wild-type inductions at ~4 hr. Since in vegetative cultures of *rad50*Δ and *rad32*Δ mutants up to ~75% of the cells do not divide (Tavassoli *et al.* 1995; Hartsuiker *et al.* 2001), we presume that the residual intact chromosomal DNA reflects these cells, which may not enter meiosis. Breakage at *mbs1* and *mbs2* was also evident in both mutants at ~3 hr and persisted for at least 8 hr after induction (Figure 2, B and C). The frequency of DNA breakage at *mbs1* was ~6% in wild-type cells (Figure 2A) and ~11% in *rad50S* mutants in which broken DNA accumulates (Young *et al.* 2002), but was only ~2% in the *rad50*Δ and *rad32*Δ mutants. We presume this reduction reflects dead cells in the mutant cultures, as noted above. We cannot exclude, however, the possibility that Rad50 and Rad32 are required for the full, wild-type level of breakage. Nevertheless, these physical analyses showed that meiotic DNA breakage, including breakage at or near *mbs1* and *mbs2*, did occur in *rad50*Δ and *rad32*Δ mutants. Little, if any, of this broken DNA was repaired in a timely manner.

These observations predict that formation of viable spores, which requires intact chromosomes, would be much reduced, as previously reported for *rad32*Δ mutants (Tavassoli *et al.* 1995), but would be rescued by a mutation blocking meiotic DNA breakage. To test this prediction, we measured the viable spore yields of *rad50*Δ and *rad32*Δ mutants with or without additional mutations in *rec6* and *rec12*, whose products are required

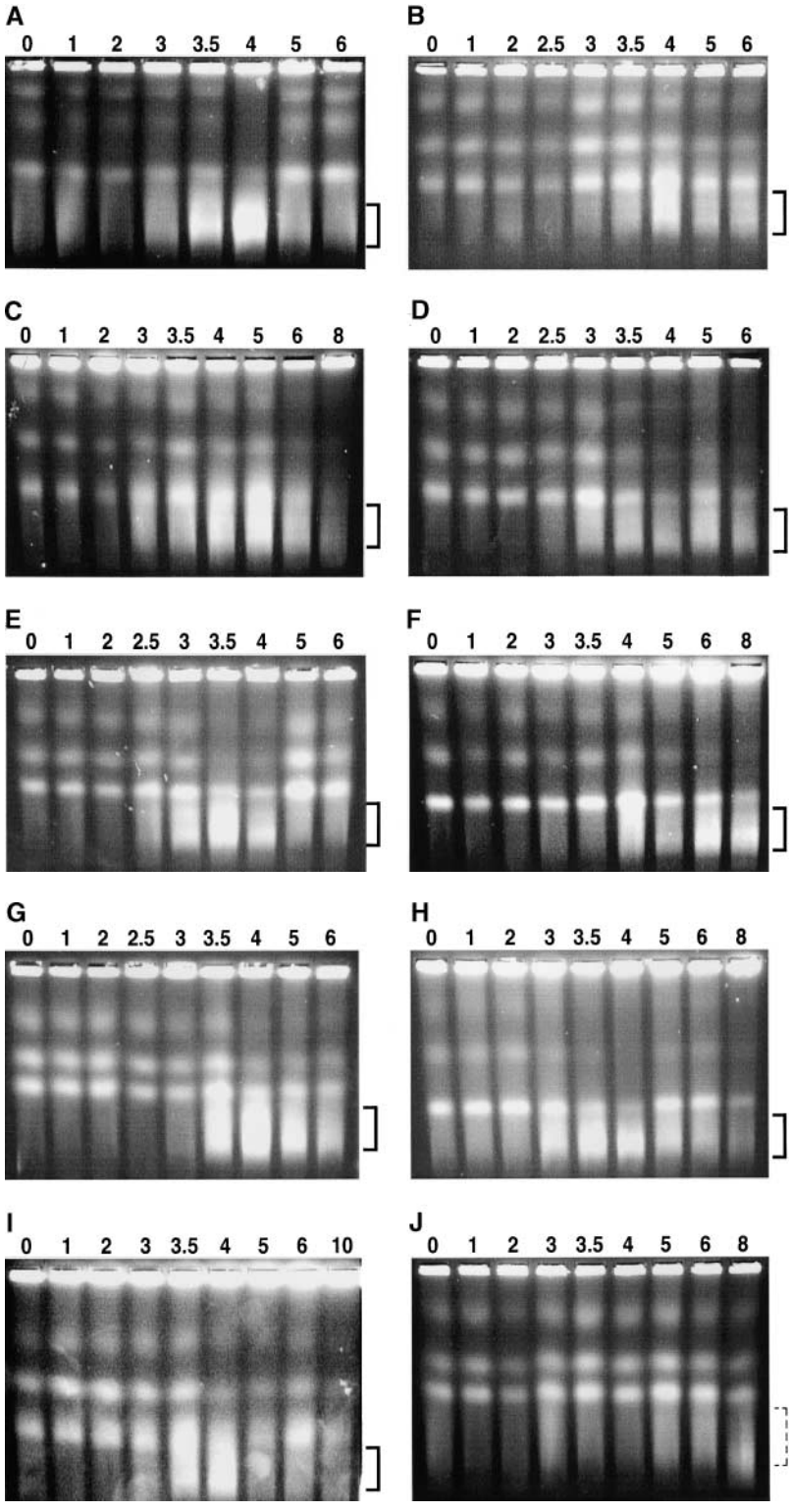

FIGURE 1.—Breakage and repair of meiotic chromosomal DNA. *S. pombe* cells with the indicated genotype were induced for meiosis and harvested after the indicated times (hours); DNA was analyzed by pulsed-field gel electrophoresis and stained with ethidium bromide as described in MATERIALS AND METHODS. The bands in the mitotic (0 hr) lanes are, from top to bottom, the loading wells and chromosomes I, II, and III (5.7, 4.6, and 3.5 Mb, respectively). The smear (bracketed) below chromosome III is meiosis-specific broken DNA that appears transiently in wild-type cells but accumulates in some mutants, such as *rad50*Δ. The broken bracket in J indicates a low level of mechanically broken DNA (not meiosis specific) that occasionally appears, such as in this induction of *mei4*Δ. (A) Wild-type (*rec*$^+$ *rad*$^+$, etc.) strain GP535. (B) *rad50*Δ strain GP3652. (C) *rad32*Δ strain GP2496. (D) *rhp51*Δ strain GP3581. (E) *dmc1*Δ strain GP3607. (F) *rhp51*Δ *dmc1*Δ strain GP4233. (G) *swi5-39* strain GP813. (H) *rad22*Δ *rti1*Δ strain GP3269. (I) *mus81*Δ strain GP3574. (J) *mei4*Δ strain GP3650. Similar results were obtained in other experiments using these or other strains containing the indicated mutations (data not shown).

for meiotic DNA breakage (CERVANTES *et al.* 2000). The results in Table 2 support these predictions. The *rad50*Δ or *rad32*Δ mutation or their combination reduced viable spore yields by a factor of ~1000, relative to wild type, and the *rec6*Δ or *rec12*Δ mutation increased the yields of the *rad50*Δ and *rad32*Δ mutants by ~70-fold. In *rec6*Δ or *rec12*Δ mutants, the yields were reduced by a factor of ~6–10 relative to wild type, at least in part because of faulty chromosome segregation (PONTICELLI and SMITH 1989; SHARIF *et al.* 2002; DAVIS and SMITH 2003). The *rec6*Δ or *rec12*Δ mutation increased the yields of the *rad50*Δ and *rad32*Δ mutants to within a factor of 2 or 3

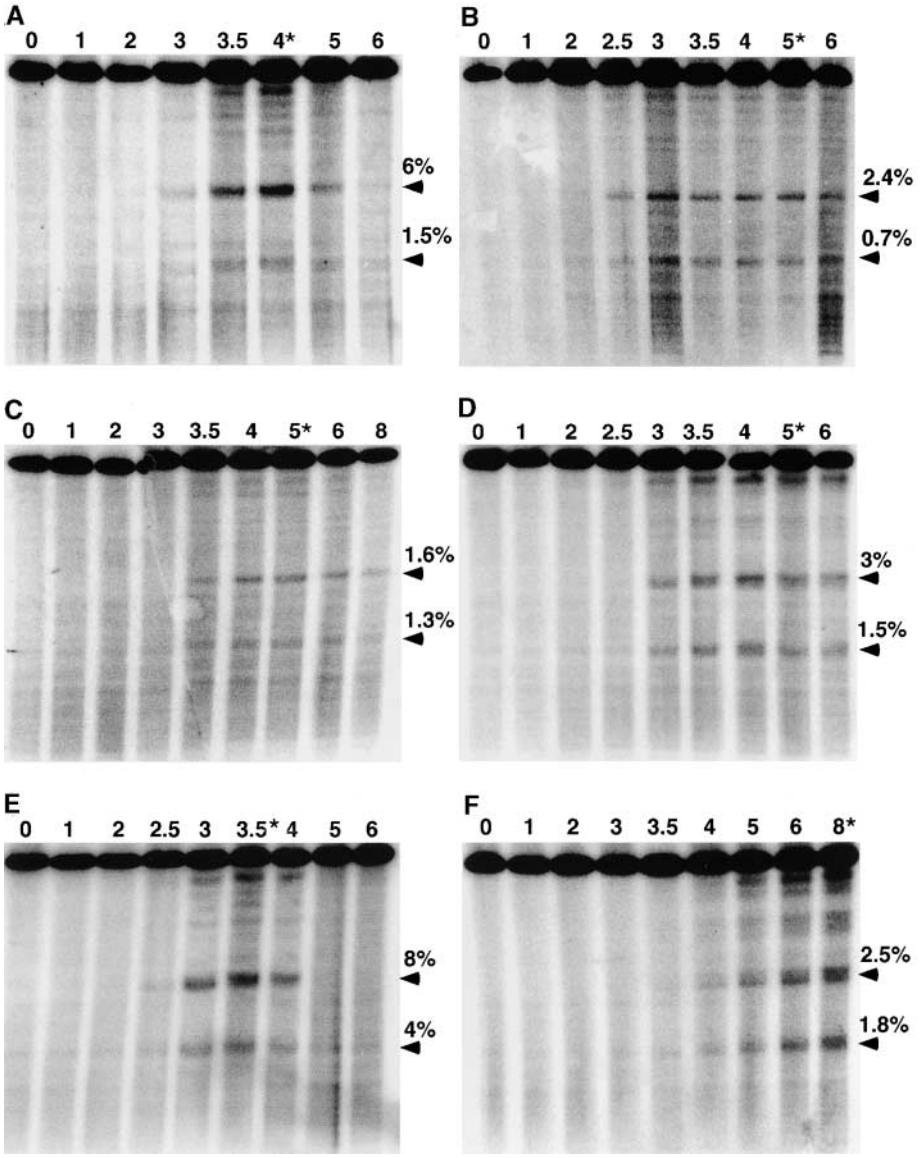

FIGURE 2.—Meiotic DNA breakage at prominent sites on the 501-kb *Not*I fragment J of chromosome I. Cells with the indicated genotype were induced for meiosis and harvested at the indicated times (hours); the DNA was digested with *Not*I and analyzed by pulsed-field gel electrophoresis and Southern blot hybridization using a probe from the left end of the *Not*I fragment J as described in MATERIALS AND METHODS. The topmost band is the unbroken *Not*I fragment J; lower bands reflect meiosis-specific breaks at the prominent meiotic break sites, such as *mbs1* and *mbs2* (top and bottom carets, respectively), described by YOUNG *et al.* (2002). The percentages of meiosis-specific DNA breakage (starred lane minus 1-hr lane) at *mbs1* and *mbs2* are indicated. The inductions correspond to those in Figure 1. (A) Wild-type (rec^+ rad^+, etc.) strain GP535. (B) *rad50*Δ strain GP3652. (C) *rad32*Δ strain GP2496. (D) *rhp51*Δ strain GP3581. (E) *dmc1*Δ strain GP3607. (F) *rhp51*Δ *dmc1*Δ strain GP4233. (G) *swi5-39* strain GP-813. (H) *rad22*Δ *rti1*Δ strain GP3269. (I) *mus81*Δ strain GP3574. (J) *mei4*Δ strain GP3650. Similar results were obtained in other experiments using these or other strains containing the indicated mutations (data not shown).

of the yields of the *rec6*Δ and *rec12*Δ single mutants; the slightly lower yields of the double mutants may reflect reduced spore germination or reduced mitotic viability of *rad50*Δ and *rad32*Δ mutants, as noted above (TAVASSOLI *et al.* 1995; HARTSUIKER *et al.* 2001).

In summary, the physical and genetic data reported here show that the *S. pombe* Rad50 and Rad32 proteins are not essential for meiotic DNA breakage but are needed for repair of these breaks.

***S. pombe* Rhp51 protein is required for meiotic DNA repair but Dmc1 protein is not:** Two homologs of the *E. coli* RecA strand-transfer protein, designated Rad51 and Dmc1, are required for repair of meiotic broken DNA in *S. cerevisiae* (ROEDER 1997). We tested the *S. pombe* mutants lacking the homologs, designated Rhp51 and Dmc1 (MURIS *et al.* 1993; SHINOHARA *et al.* 1993; FUKUSHIMA *et al.* 2000), for meiotic DNA breakage and repair. In both *rhp51*Δ and *dmc1*Δ mutants bulk DNA breakage occurred at ~3 hr (Figure 1, D and E), as in wild-type cells (Figure 1A). In the *dmc1*Δ mutant repair occurred at ~5 hr, as in wild-type cells, but in the *rhp51*Δ mutant broken DNA remained, as in the *rad50*Δ and *rad32*Δ mutants discussed above. ZENVIRTH and SIMCHEN (2000) also concluded that meiotic DNA is broken but not repaired in an *rhp51*Δ mutant, and SHIMADA *et al.* (2002) also showed that whole chromosomes are broken and repaired with similar kinetics in $dmc1^+$ and *dmc1*Δ strains. Analysis of *Not*I-digested DNA revealed a pattern similar to that of bulk chromosomal DNA: in both mutants DNA was broken at *mbs1* and *mbs2*, and it was repaired in the *dmc1*Δ mutant but not in the *rhp51*Δ mutant (Figure 2, D and E). The *rhp51*Δ *dmc1*Δ double mutant behaved much like the *rhp51*Δ single mutant (Figures 1F and 2F); breakage in the double mutant was slightly later than that in either single mutant, perhaps because DNA replication was later in the double mutant in this experiment (data not shown). These results show that Rhp51 plays a major role in the

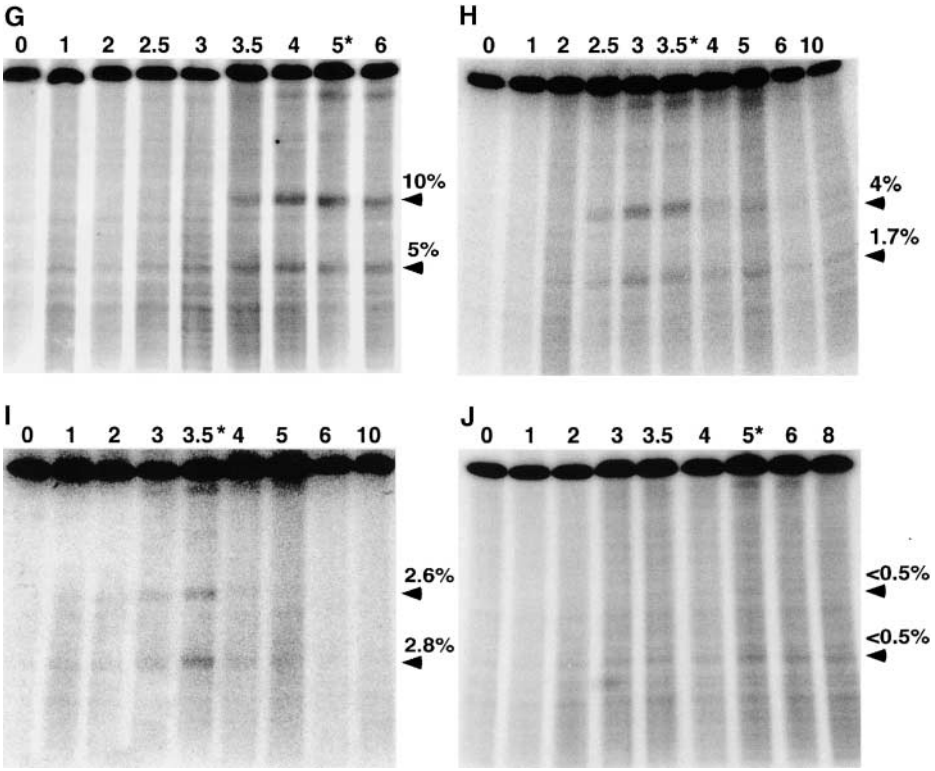

FIGURE 2.—*Continued.*

repair of meiotic broken DNA, but Dmc1 plays at most a minor role (see DISCUSSION).

The small protein Swi5 is required for meiotic DNA repair: *S. pombe swi5* mutants were isolated on the basis of their reduced frequency of mating-type switching (GUTZ and SCHMIDT 1985). The *swi5* gene encodes a polypeptide of 85 amino acids with ~20% amino acid sequence identity to *S. cerevisiae* Sae3, which is required for meiotic DNA break repair (MCKEE and KLECKNER 1997; AKAMATSU *et al.* 2003; see DISCUSSION). The frequencies of intragenic and intergenic meiotic recombination in *swi5* mutants are also reduced by a factor of ~5 for the intervals reported (SCHMIDT *et al.* 1987; DEVEAUX *et al.* 1992; SCHMIDT 1993). Consequently, we examined meiotic DNA breakage and repair in the *swi5-39* mutant, which bears a nonsense mutation in codon 38 (AKAMATSU *et al.* 2003). Bulk chromosomal DNA breakage and breakage at *mbs1* and *mbs2* occurred at ~3.5 hr (Figures 1G and 2G), approximately as in wild type. Much of the broken DNA persisted for at least 12 hr, and there was little, if any, recovery of intact chromosomal DNA by this time (Figures 1G and 2G; data not shown); repair in wild-type cells was essentially complete by 5 hr (Figures 1A and 2A). Thus, Swi5 is required for repair, but not for formation, of meiotic DNA breaks.

The deficiency of meiotic DNA repair in the *swi5-39* mutant appeared similar to that of *rad50*Δ and *rad32*Δ mutants. Viable spore yield is strongly reduced in *rad50*Δ and *rad32*Δ mutants (TAVASSOLI *et al.* 1995; HARTSUIKER *et al.* 2001; Table 2), but, paradoxically, spore viability is as high in *swi5* mutants as in wild type under the conditions reported (malt extract medium at 25°; SCHMIDT 1993). To address this paradox, we measured meiotic recombination and spore viability under the conditions used for meiotic DNA break determination (EMM2 at 34°) and, for comparison, at the standard temperature of 25°. The results in Table 3 show that, with respect to *ade6* intragenic recombination and viable spore yield, the *swi5-39* mutant is not strongly temperature sensitive. In standard matings of *pat1*$^+$ haploids, the *swi5-39* mutation reduced *ade6* recombination by a factor of ~10, and the viable spore yields by a factor of ~3, relative to *swi5*$^+$. Spore viability, measured after micromanipulation of ascal tetrads or random spores, was also reduced by a factor of ~3 in *swi5-39* relative to *swi5*$^+$. In meiotic inductions of *pat1-114* haploids, viable spore yields were similar in *swi5*$^+$ and *swi5-39* mutants, ~1 viable spore/100 cells induced, as reported previously for *swi5*$^+$ cells (IINO and YAMAMOTO 1985). Comparing *pat1*$^+$ strains, these levels of viable spore yield and spore viability are much higher than those of *rad50*Δ and *rad32*Δ mutants (TAVASSOLI *et al.* 1995; HARTSUIKER *et al.* 2001; Table 2), even though the level of broken DNA accumulated in meiosis appears at least as high in the *swi5-39* mutant as in *rad50*Δ or *rad32*Δ mutants (Figures 1 and 2). Perhaps in *swi5* mutants, but not in *rad50*Δ or *rad32*Δ mutants, broken DNA is repaired late in meiosis or shortly after spore germination, thereby giving rise to higher levels of viable spores.

Rad22 and Rti1, two Rad52 homologs, are required for meiotic DNA repair: The *S. cerevisiae* Rad52 protein is required for repair of meiotic DNA breaks; in its

TABLE 2

Genetic evidence that Rad32 and Rad50 are not required for meiotic DNA breakage: *rec* mutations blocking DNA breakage suppress the low viable spore yield of *rad32* and *rad50* deletion mutants

	Relative viable spore yield (%)[b]		
rad genotype[a]	*rec*$^+$	*rec6*Δ	*rec12*Δ
+	100	15.7 ± 1.3 (8)	9.9 ± 0.7 (8)
*rad32*Δ	0.094 ± 0.01 (8)	6.5 ± 1.0 (4)	3.8 ± 0.5 (4)
*rad50*Δ	0.062 ± 0.008 (8)	4.4 ± 1.2 (4)	4.2 ± 0.8 (4)
*rad32*Δ *rad50*Δ	0.050 ± 0.002 (4)	ND	3.8 ± 0.3 (4)

ND, not determined.

[a] Alleles were *rad32::ura4*$^+$ (Tavassoli *et al.* 1995), *rad50::kanMX* (Hartsuiker *et al.* 2001), *rec6-151::LEU2* (Lin and Smith 1994), and *rec12-152::LEU2* (Lin and Smith 1994) and were homozygous mutant or wild type as indicated. Three pairs of parental strains were used for the *rad*$^+$ *rec*$^+$ crosses, two for the *rad50*Δ *rec*$^+$ crosses, and two for the *rad*$^+$ *rec6*Δ crosses. Complete genotypes and genealogies are available upon request.

[b] Mating and meiosis were on SPA agar at 25°. Viable spore yields are expressed relative to wild-type matings done concurrently, which produced 5.8 ± 0.5 (n = 16) viable spores per viable cell (of the less numerous parent) added to the mating mixture. This yield is slightly greater than the theoretical four spores per haploid cell (of one parent) because of slight residual growth of cells on the sporulation medium. Data are the means ± SEM from the number of independent matings indicated in parentheses.

absence the broken DNA is hyperresected from its ends (Soustelle *et al.* 2002). *S. pombe* contains two genes, designated *rad22* (or *rad22A*) and *rti1* (or *rad22B*), whose products over extensive portions have, respectively, ~33 and ~37% amino acid sequence identity to Rad52 (Ostermann *et al.* 1993; Suto *et al.* 1999; van den Bosch *et al.* 2001). Disruption of both *S. pombe* genes strongly reduces spore viability; single gene disruptions have only a mild effect (van den Bosch *et al.* 2001). We therefore examined the *rad22*Δ *rti1*Δ double mutant for meiotic DNA breakage and repair.

In the *rad22*Δ *rti1*Δ double mutant, meiotic DNA breakage occurred at ~3 hr, as in wild type, as assayed by analysis of either bulk chromosomal DNA (Figure 1H) or DNA broken at *mbs1* or *mbs2* (Figure 2H). Some broken DNA persisted beyond the time of its repair in wild type at ~4.5 hr; bulk chromosomal DNA fragments were visible as late as 8 hr (Figure 1H). Intact DNA reappeared at ~5 hr in the *rad22*Δ *rti1*Δ mutant at low level (Figure 1H) but in wild type at high level (Figure 1A). DNA fragments broken at *mbs1* or *mbs2* became less abundant by 6–10 hr (Figure 2H); this disappearance may reflect degradation or repair of the broken DNA or both. We conclude that neither Rad22 nor Rti1 is required for meiotic DNA breakage, but one or both proteins is required for high-level repair.

Mus81 is required after meiotic DNA breakage: The *S. pombe* Mus81•Eme1 protein complex is required for production of viable spores and can cleave branched DNA molecules (Boddy *et al.* 2001; Gaillard *et al.* 2003; Osman *et al.* 2003). Like *rad50* and *rad32* mutants (Table 2), *mus81* and *eme1* mutants produce few viable spores, and introduction of a *rec6* or *rec12* mutation increases viable spore yields to the level of the *rec* mutants (Boddy *et al.* 2001). These are the properties expected of mutants lacking an enzyme that cleaves joint molecules. As expected from this view, DNA breakage in a *mus81*Δ mutant occurred at the normal time (~3 hr), and the broken DNA largely disappeared at approximately the normal time (~5 hr; Figures 1I and 2I). Intact chromosomal DNA, however, did not consistently reappear in all experiments; the DNA may have been degraded, or it may have been in branched structures that failed to enter the gel. (We noted that in some inductions of the *mus81*Δ mutant, like those of the *rad50*Δ and *rad32*Δ mutants, intact chromosomal DNA did not entirely disappear, perhaps due to dead cells in some cultures, as noted previously.)

A meiosis-specific transcription factor, Mei4, is required for meiotic DNA breakage: In *S. cerevisiae*, the midmeiosis transcription factor Ndt80 is required for the proper completion of repair, but not formation, of broken meiotic DNA; presumably, the product of an unknown gene activated by Ndt80 resolves joint molecules into crossover recombinant molecules (Xu *et al.* 1995; Allers and Lichten 2001). In *S. pombe* the Mei4 forkhead-like transcription factor is induced early in meiosis, beginning at ~2 hr but reaching its maximum level at ~5 hr, and activates genes whose products function later in meiosis, which, for most genes, is >4 hr after induction (Horie *et al.* 1998; Abe and Shimoda 2000). Because of this functional similarity, we examined meiotic DNA in a *mei4*Δ mutant. Bulk chromosomal DNA remained largely intact for up to 8 hr after induction; little or no broken DNA was visible by assay of either bulk chromosomal DNA or DNA broken at *mbs1* or *mbs2* (Figures 1J and 2J). DNA replication, assayed by flow cytometry (Li and Smith 1997), was complete and occurred at the normal time, ~2 hr after induction (data not shown), as reported previously for

TABLE 3

***swi5* mutation reduces meiotic recombination and spore viability**

	Relative *ade6*$^+$ recombinant frequency[a]	
Temperature	*swi5*$^+$	*swi5-39*
25°	100	14 ± 3
34°	86 ± 4	7.7 ± 0.3
	Relative viable spore yield[b]	
Temperature	*swi5*$^+$	*swi5-39*
25°	100	39 ± 7
34°	107 ± 7	23 ± 0.5
34°[c]	100	92 ± 28
	Spore viability[d]	
Temperature	*swi5*$^+$	*swi5-39*
34°	0.95 (0.81)	0.36 (0.25)

[a] Heterothallic *ade6-M26* and *ade6-52* strains with the indicated *swi5* genotype were mated on EMM2 at the indicated temperature. The *ade6*$^+$ recombinant frequencies, determined as described in MATERIALS AND METHODS, are expressed relative to that for *swi5*$^+$ matings done concurrently at 25°; this value was 9.3 ± 1.1 Ade$^+$/10^3 viable spores. Data are means ± SEM (n = 5).

[b] Viable spore yields, determined as in Table 2, are expressed relative to that for *swi5*$^+$ at 25°, which produced 4.4 ± 0.6 viable spores per viable cell. Data are from the matings described in footnote *a*.

[c] In these experiments haploid *pat1-114* strains were induced for meiosis by the procedure for assaying DNA breakage and repair. Viable spore yields, at 24 hr, are expressed relative to that for *swi5*$^+$, which produced 0.010 ± 0.0015 (n = 5) viable spores per viable cell induced.

[d] Four-spored asci (10 for *swi5*$^+$ and 11 for *swi5-39*) from matings on EMM2 were dissected, and the fraction of spores that produced a visible colony on YEA rich medium recorded. In parentheses is the fraction of 52 random spores, after standard glusulase and EtOH treatment, that produced a visible colony.

wild-type cells (CERVANTES *et al.* 2000; YOUNG *et al.* 2002). H. MURAKAMI (personal communication) has also observed that bulk chromosomal DNA remains largely intact in a *mei4* mutant during meiosis. We conclude that Mei4, or more likely the product(s) of one or more genes controlled by it, is required for meiotic DNA breakage but for not replication.

DISCUSSION

We have reported here both similarities and differences in the proteins required for meiotic DNA breakage and repair in *S. pombe* (*Sp*) and those required in the distantly related yeast *S. cerevisiae* (*Sc*). Some proteins with extensive amino acid sequence identity appear to have similar or identical functions in the two yeasts. For example, the *Sp*Rec12 and *Sc*Spo11 proteins share an essential Tyr in a conserved sequence motif and are essential for meiotic DNA breakage; the essential Tyr is likely at the active site (KEENEY *et al.* 1997; CERVANTES *et al.* 2000; KEENEY 2001). Similarly, the *Sp*Rhp51 and *Sc*Rad51 proteins resemble the *E. coli* RecA strand transfer protein and are required for repair of meiotic broken DNA (ABOUSSEKHRA *et al.* 1992; SHINOHARA *et al.* 1992, 1993; MURIS *et al.* 1993; Figures 1D and 2D). Thus, the basic mechanisms of meiotic DNA breakage by topoisomerase-like cleavage and of repair by strand exchange with homologous DNA appear to be conserved between these two yeasts and possibly most or all eukaryotes (KEENEY 2001).

Other proteins, however, with similar sequences have different functions in the two yeasts (Table 4); examples include the *Sp*Rad50•Rad32•Nbs1 and the *Sc*Rad50•Mre11•Xrs2 complexes discussed below. In addition, some proteins required for meiotic DNA breakage or repair have been identified in one yeast but not in the other (Table 5), in spite of their complete or nearly complete genome sequences being known (GOFFEAU *et al.* 1996; WOOD *et al.* 2002). Below, we discuss these similarities and differences and their implications for meiotic recombination.

Proteins required for meiotic DNA breakage: At least three proteins required for meiotic DNA breakage appear to be conserved between *S. pombe* and *S. cerevisiae*. These are *Sp*Rec12 and *Sc*Spo11; *Sp*Rec7 and *Sc*Rec114; and *Sp*Rec14 and *Sc*Ski8 (also called Rec103). Mutations in these genes abolish meiotic DNA breakage and recombination (reviewed in DAVIS and SMITH 2001; KEENEY 2001). As noted above, Spo11 and its homologs likely contain the active site that cleaves the DNA; the other proteins may form with Spo11 a complex essential for its activity. Additional proteins apparently unique to each yeast are also essential for meiotic breakage and recombination (Table 5): these include Rec6, Rec10, and Rec15 in *S. pombe* and Rec102, Rec104, Rec107 (also called Mer2), and Mei4 in *S. cerevisiae*. (*Sp*Mei4 and *Sc*Mei4 are not related.) The absence of identifiable homologs for these proteins in interspecies comparisons may reflect a lack of amino acid sequence conservation for proteins with conserved function. Alternatively, some aspect of breakage and recombination, such as regulation of the distribution of breakage and recombination along chromosomes, may differ in these two yeasts. This hypothetical difference in regulation may be related to the presence of a full-fledged synaptonemal complex and crossover interference in *S. cerevisiae* but to their absence in *S. pombe* (KOHLI and BÄHLER 1994). Consistent with this suggestion, *Sc*Hop1 and Red1, components of the synaptonemal complex, are required for high-level breakage and recombination in *S. cerevisiae* (KEENEY 2001), but *S. pombe* lacks obvious homologs.

A major difference in the proteins required for meiotic DNA breakage is the requirement for the *Sp*Rad50•Rad32•Nbs1 and *Sc*Rad50•Mre11•Xrs2 complexes. [We

TABLE 4

Summary of meiotic DNA breakage and repair in *S. pombe* mutants studied here and in related *S. cerevisiae* mutants

S. pombe			*S. cerevisiae*		
Mutant	Breakage	Repair	Mutant	Breakage	Repair
rad50	+	−	*rad50*	−	−
rad32	+	−	*mre11*	−	−
rhp51	+	−	*rad51*	+	−
dmc1	+	+	*dmc1*[a]	+	+ or −
swi5	+	−	*sae3*	+	−
rad22 rti1	+	−[b]	*rad52*	+	−
mus81	+	−	*mus81*	+	+
mei4[c]	−	NA	*ndt80*[d]	+	−

+, breakage or repair occurs in the indicated mutant; −, breakage or repair does not occur or is significantly reduced. NA, not applicable, because repair has not been assessed in the absence of normal breakage.

[a] *dmc1* mutations have a more severe phenotype in derivatives of strain SK1 than in derivatives of another strain (see DISCUSSION).

[b] Repair at low level occurs in the *rad22 rti1* mutant (see DISCUSSION).

[c] The *S. pombe* Mei4 protein is not a homolog of the *S. cerevisiae* Mei4 protein.

[d] Ndt80 is not structurally related to Mei4 (see DISCUSSION), nor is there evidence for homology between these proteins.

assume that the *Sp* polypeptides form a functional complex, as do the *Sc* and human proteins. After completion of this work, CHAHWAN *et al.* (2003) and UENO *et al.* (2003) reported that the *Sp*Nbs1 polypeptide appears to be functionally similar to *Sc*Xrs2, although they share only very limited amino acid sequence identity. The *rad50*, *rad32*, and *nbs1* single, double, and triple mutants have nearly identical sensitivities to DNA-damaging agents, and tagged versions of Nbs1 and Rad32 co-immunoprecipitate.] The *Sc* complex is required for meiotic DNA breakage (ALANI *et al.* 1990; JOHZUKA and OGAWA 1995), but the *Sp* complex is not (Figure 1, B and C; Figure 2, B and C). Similarly, the *Sc* complex is required for nonhomologous end-joining (PAQUES and HABER 1999), but the *Sp* complex is not (MANOLIS *et al.* 2001). Both complexes are required, however, for mitotic and meiotic ds DNA break repair, as discussed below. As expected, *S. pombe rad50*Δ and *rad32*Δ mutants form very few viable spores, but this phenotype is largely suppressed by a *rec6*Δ or *rec12*Δ mutation (Table 2), each of which blocks meiotic DNA breakage (CERVANTES *et al.* 2000; YOUNG *et al.* 2002). In the basidiomycete *Coprinus cinereus* Rad50 also appears dispensable for meiotic DNA breakage: *rad50* mutants arrest in meiosis I prophase, but this arrest is alleviated by a *spo11* mutation, and gamma-irradiation of the *rad50 spo11* double mutant restores the arrest (S. ACHARYA and M. ZOLAN, personal communication).

*Sc*Mre11 binds to meiotic chromosomes in a Spo11-dependent manner, and it has been speculated that *Sc*Rad50•Mre11•Xrs2 must be in place for it to modify chromatin or to effect subsequent repair before Spo11 makes DNA breaks (BORDE *et al.* 2004). If so, *Sp*Rec12 is apparently not regulated in the same way by Rad50 and Rad32, since Rec12-dependent DNA breakage occurs in the absence of Rad50 or Rad32. *S. pombe* may represent a simpler scheme in which meiotic DNA breakage is not coupled to the potential for repair.

Proteins required for meiotic DNA break repair: Several proteins required for high levels of meiotic DNA break repair appear to be conserved: these include

TABLE 5

Meiotic DNA breakage and repair proteins identified in one yeast but not obviously recognizable in the other

	Required for meiotic DNA	
Protein[a]	Breakage	Repair
Identified in *S. pombe*		
Rec6	Y[b]	NA
Rec10	Y	NA
Rec15	Y	NA
Identified in *S. cerevisiae*		
Rec102	Y	NA
Rec104	Y	NA
Rec107 (Mer2)	Y	NA
Mei4[c]	Y	NA
Hop1	Y[d]	NA
Red1	Y[d]	NA
Sae2 (Com1)	N	Y

NA, not applicable, because repair has not been assessed in the absence of normal breakage.

[a] For a protein identified in one yeast, a BLASTp search revealed only proteins with very limited amino acid sequence identity ($P > 0.01$) in the other yeast.

[b] The indicated protein is required (Y) or is not required (N) for wild-type levels of meiotic DNA breakage or repair. For references see DAVIS and SMITH (2001) and ROEDER (1997).

[c] *S. cerevisiae* Mei4 protein is not a homolog of the *S. pombe* Mei4 protein.

[d] Breakage is reduced but not eliminated in these mutants.

*Sp*Rad50•Rad32 and *Sc*Rad50•Mre11; *Sp*Rhp51 and *Sc*Rad51; and *Sp*Rad22 and Rti1 and *Sc*Rad52. In *S. cerevisiae* Rad50•Mre11•Xrs2 is required for resection of ds DNA ends to produce 3′-ended ss tails, to which Rad51 binds and promotes strand exchange with a homologous duplex (Roeder 1997; Haber 1998). In assays using purified components, Rad52 aids Rad51 in formation of joint molecules (Sung *et al.* 2000); in cells, Rad52 appears to promote binding of Rad51 to ss DNA adjacent to a ds break (Sugawara *et al.* 2003; Wolner *et al.* 2003). In *rad51* and *rad52* mutant meiotic cells, ds DNA ends are hyperresected; presumably, joint molecules are not formed (Shinohara *et al.* 1992; Sousteelle *et al.* 2002). Resection of ds DNA ends and formation of joint molecules have not, to our knowledge, been assessed in *S. pombe*. Nevertheless, the persistence of broken DNA in the corresponding *S. pombe* mutant meiotic cells (Figures 1H and 2H) and the extensive amino acid sequence similarities between the corresponding proteins are consistent with the view that these proteins have similar functions in the two yeasts.

A second RecA homolog, Dmc1, may have a different role in the two yeasts. *S. pombe dmc1* mutants repaired broken DNA at the normal time (Figures 1E and 2E), have high spore viability (~75%), and have only modestly reduced meiotic recombinant frequencies (~30% of wild type; Fukushima *et al.* 2000; Grishchuk and Kohli 2003). In *S. cerevisiae dmc1* mutants derived from strain SK1, meiotic ds DNA ends are hyperresected, joint molecules and recombinant molecules are made at only low levels, and cells arrest at the pachytene stage of meiosis (Bishop *et al.* 1992; Schwacha and Kleckner 1994). *S. cerevisiae dmc1* mutants derived from a different strain, however, progress through meiosis and form spores in which the recombinant frequency is ~40% of wild type (Rockmill 1995). Thus, Dmc1 appears to play a major role in meiotic recombination in some *S. cerevisiae* strains but only a minor role in other *S. cerevisiae* strains and in *S. pombe*. Nevertheless, in both *S. cerevisiae* and *S. pombe* transcription of the *dmc1* gene is very strongly induced early in meiosis (Bishop *et al.* 1992; Fukushima *et al.* 2000), suggesting an important role in meiosis. Since *S. pombe dmc1 rhp51* double mutants have very low viable spore yields and recombinant frequencies (Grishchuk and Kohli 2003) and accumulate broken meiotic DNA (Figures 1F and 2F), Dmc1 appears to provide an alternative to Rhp51 for DNA strand exchange, as in *S. cerevisiae* (Roeder 1997). The role of the seemingly minor Dmc1 alternative in *S. pombe* remains to be elucidated.

The small protein Swi5 appears to be essential for the timely repair of meiotic broken DNA (Figures 1G and 2G). In *swi5* mutants intragenic and intergenic recombinants, likely reflecting gene conversions and crossovers, respectively, arise at frequencies 5–10 times lower than those in *swi5*$^{+}$ cells (Schmidt *et al.* 1987; DeVeaux *et al.* 1992; Schmidt 1993; Table 3). Swi5 thus plays a major role in the repair of meiotic broken DNA and recombination. Mating-type switching in *S. pombe* is a type of homologous recombination that involves a ss DNA break, which may be converted to a ds break by replication (Beach 1983; Arcangioli 1998; Arcangioli and de Lahondes 2000). *swi5* mutants have nearly normal levels of broken DNA at the *mat1* locus (Egel *et al.* 1984) but switch at low frequencies (Gutz and Schmidt 1985). These low-frequency recombinants appear to have the normal structure. Thus, Swi5 appears to be important but not absolutely essential for repairing broken DNA in both meiotic and mating-type-switching recombination.

The activity of the remarkably small Swi5 protein—85 amino acids (Akamatsu *et al.* 2003)—remains to be elucidated, but recent evidence suggests that it may be involved in DNA strand exchange. Swi5 indirectly interacts physically with Rhp51 via Sfr1 (Akamatsu *et al.* 2003). Swi5 shares ~20% sequence identity with a 102-amino-acid form of *S. cerevisiae* Sae3, predicted from conceptual RNA splicing and translation (V. Wood, personal communication). *S. cerevisiae dmc1*, *sae3*, and *dmc1 sae3* double mutants have similar phenotypes: in the SK1 background they accumulate hyperresected meiotic broken DNA and arrest in the first meiotic prophase (McKee and Kleckner 1997). The different phenotypes of *S. pombe rhp51*, *dmc1*, and *swi5* mutants suggest, within this framework, complex interactions among strand exchange activities.

The Mus81•Eme1 complex of *S. pombe* has many of the properties expected for a Holliday junction resolvase. The protein cleaves Holliday junctions at or near their branch point with a preference for junctions already containing a nick (Boddy *et al.* 2001; Gaillard *et al.* 2003). Mutants lacking either subunit form few viable spores; a *rec6* or *rec12* mutation increases spore viability to the level of the single *rec* mutants (Boddy *et al.* 2001), paralleling the phenotypes of the *rad50* and *rad32* mutants (Table 2). In the *mus81* mutant, meiotic DNA breakage occurred at the normal time; the broken DNA disappeared, but intact chromosomal DNA did not consistently reappear (Figures 1I and 2I). This is the behavior expected in the absence of resolution of joint molecules. Crossing over, but not gene conversion, is severely reduced in *mus81* mutants (Osman *et al.* 2003; Smith *et al.* 2003). These data are thus consistent with Mus81•Eme1 being an essential Holliday junction resolvase in *S. pombe*. The corresponding protein in *S. cerevisiae*, Mus81•Mms4, also cleaves branched DNA molecules near their branch point, but mutants lacking the protein have nearly normal meiotic recombinant frequencies among the spores produced; few cells, however, produce spores (Interthal and Heyer 2000; de los Santos *et al.* 2003). Thus, *Sp*Mus81•Eme1 plays a more critical role in meiotic recombination than does *Sc*Mus81•Mms4. *S. pombe* may have a single pathway for resolution of joint molecules into recombinants, and *S. cerevisiae*

may have two or more pathways (de los Santos *et al.* 2003).

The *S. pombe* Rad22 and Rti1 proteins are required for high levels of meiotic DNA break repair (Figures 1H and 2H), as is the related *S. cerevisiae* Rad52 protein (Sousstelle *et al.* 2002). As expected, the *S. pombe* double mutant has very low spore viability: ~3% compared to 92% for wild type in the same study (van den Bosch *et al.* 2001). Curiously, the meiotic *ade6* intragenic recombinant frequency among the few viable spores is near that of wild type (van den Bosch *et al.* 2002). Perhaps a small subpopulation of cells repairs broken DNA normally, as suggested by the reappearance of a low level of intact DNA in the *rad22 rti1* mutant (Figure 1H); this subpopulation may form viable spores with normal recombinant frequencies. Mitotic *S. pombe* cells also have a subpopulation with a higher-than-average frequency of recombination, and the relative size of this subpopulation is increased in *rad2* mutants (Grossenbacher-Grunder 1985). The distinguishing features of these hypothetical subpopulations are unknown.

Transcriptional regulation of genes for meiotic DNA breakage and repair: In both *S. pombe* and *S. cerevisiae* ~10% of all genes are induced during meiosis by a complex interplay of physiological changes that signal starvation and transcriptional regulators that in part signal diploidy (Chu *et al.* 1998; Mata *et al.* 2002). Many of the genes whose products are involved in meiotic DNA breakage and repair are induced. In *S. pombe* all of the genes whose products are known to be required for meiotic DNA breakage (*rec6*, *-7*, *-8*, *-10*, *-11*, *-12*, *-14*, and *-15* and *mei4*) are induced during meiosis (Fox and Smith 1998; Horie *et al.* 1998; Mata *et al.* 2002); of those tested (*rec6*, *-7*, *-8*, *-10*, *-11*, and *-15*), induction of all but *rec10* depends upon the Rep1 (also called Rec16) protein (Li and Smith 1997; Ding and Smith 1998), which likely forms a complex with the Cdc10 transcriptional activator (Sugiyama *et al.* 1994). As expected, *rep1* (*rec16*) mutants are strongly deficient in meiotic recombination (DeVeaux *et al.* 1992; Ding and Smith 1998); meiotic DNA breakage has not been tested in *rep1* (*rec16*) mutants but is expected not to occur owing to the failure of induction of genes such as *rec6*.

A second *S. pombe* transcriptional activator, Mei4, is required for the induction of genes midway through meiosis and for completion of the first meiotic division (Horie *et al.* 1998; Abe and Shimoda 2000). Mei4 is not required for the induction of the *rec* genes tested (*rec6*, *-7*, *-8*, *-10*, and *-12*; Abe and Shimoda 2000), however, so we expected that breaks would be made but perhaps not repaired; *S. cerevisiae* mutants lacking the structurally unrelated midmeiosis transcriptional activator Ndt80 make breaks but not crossover recombinant DNA (Xu *et al.* 1995; Allers and Lichten 2001). Instead, we found that breaks were not made in *S. pombe mei4* mutants (Figures 1J and 2J). This result implies that Mei4 controls the expression of one or more early meiotic genes required for DNA breakage. Like Ndt80, it may also control one or more later meiotic genes for the proper repair of broken DNA.

The results discussed here illustrate the diversity of proteins required for meiotic DNA breakage and repair and suggest divergence of the mechanism or control of meiotic recombination. Such divergence is concordant with the divergence of other aspects of meiosis among eukaryotes—for example, the presence or absence of a synaptonemal complex and the arrest of meiosis for years in some organisms (Moens 1987). Further investigations are required to reveal the universal features of DNA metabolism during meiotic recombination.

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