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Swi5 Acts in Meiotic DNA Joint Molecule Formation in *Schizosaccharomyces pombe*

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ABSTRACT

Previously isolated *Schizosaccharomyces pombe swi5* mutants are defective in mitotic mating-type switching and in repair of meiotic recombination-related DNA double-strand breaks. Here, we identify the *swi5* gene, which encodes an 85-amino-acid polypeptide, similar to Sae3 of *Saccharomyces cerevisiae*, with an N-terminal predicted coiled-coil domain. A *swi5* complete deletion mutant had normal mitotic growth rate but was hypersensitive to DNA-damaging agents and defective in mating-type switching. In meiosis, recombinant frequencies were reduced by a factor of ~10. The *swi5* deletion strongly reduced the viable spore yields of mutants lacking Rhp55 or Rhp57, proteins thought to aid joint molecule formation. Furthermore, the *swi5* deletion strongly suppressed the low viable spore yield of mutants lacking Mus81•Eme1, which resolves joint molecules such as Holliday junctions. These and previous results indicate that the small Swi5 polypeptide acts in a branched pathway of joint molecule formation to repair meiotic DNA breaks.

THE repair of DNA strand breaks is crucial for the life of cells, since unrepaired broken DNA is likely to misegregate at cell division and lead to aneuploidy and consequent sickness or death. DNA breaks can arise from accidents during replication or transcription or from exogenous DNA-damaging agents such as UV or ionizing irradiation. The faithful repair of DNA double-strand (ds) breaks appears to occur most frequently by homologous recombination, which requires the formation of joint molecules between the broken DNA and an intact homolog. In special circumstances cells use programmed DNA breaks to stimulate recombination. For example, in the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* both mating-type switching and meiotic recombination are initiated by programmed DNA breaks (Haber 1998b; Davis and Smith 2001; Arcangioli and Thon 2003). As expected, certain mutants of these species are deficient in both processes and in the repair of damaged DNA.

Ten linkage groups of mutations that reduce the frequency of mating-type switching in *S. pombe* have been described (Gutz and Schmidt 1985). Mutants altered in one of these groups, *swi5*, are also hypersensitive to UV and ionizing irradiation and deficient in meiotic recombination (Schmidt *et al.* 1987, 1989). During meiosis *swi5* mutants make DNA breaks but do not efficiently repair them (Young *et al.* 2004). During mitotic growth a DNA lesion at the *mat1* locus that is essential for mating-type switching is made at normal level in *swi5* mutants, but this lesion only inefficiently leads to switching (Egel *et al.* 1984). Thus, Swi5 is important for DNA break repair in both meiosis and mitosis.

Repair of DNA breaks by homologous recombination proceeds in steps via a joint molecule intermediate. Single-strand (ss) DNA is produced at the break via DNA unwinding by a helicase or digestion by a nuclease. The ss DNA pairs and undergoes strand exchange with an intact homologous DNA molecule to form a joint molecule, such as a D-loop or a Holliday junction. This step is promoted by bacterial RecA protein or its eukaryotic homolog Rad51; these proteins are aided by others, such as *S. cerevisiae* Rad52, Rad55, and Rad57 (Sung *et al.* 2000). Joint molecules are resolved into separate molecules by special enzymes; for example, Holliday junctions are resolved by the bacterial RuvC protein or, in *S. pombe*, the Mus81•Eme1 complex (Boddy *et al.* 2001; Gaillard *et al.* 2003; Osman *et al.* 2003). Remaining ss nicks and gaps are sealed by DNA polymerases and ligases.

We report here the identification of *swi5*, which encodes a remarkably small protein. Akamatsu *et al.* (2003) independently identified the *swi5* gene and studied its role in mitotic DNA repair. Results presented here indicate that Swi5 is involved in DNA strand exchange, *i.e.*, before joint molecule resolution by Mus81•Eme1 during meiotic recombination.

MATERIALS AND METHODS

Strains and plasmids: Strains and their genotypes are listed in Table 1. Plasmids pCE1 and pCE2 were constructed by insert-

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TABLE 1

***S. pombe* strains**

Strain	Genotype	Source or reference
GP13	*h*$^-$ *ade6-52*	Ponticelli and Smith (1989)
GP24	*h*$^+$ *ade6-M26*	Ponticelli and Smith (1989)
GP50	*h*^{90}	A. Ponticelli[a]
GP289	*h*$^-$ *ade6-52 rec10-109*	Ponticelli and Smith (1989)
GP583	*h*$^-$ *ade6-52 swi5-134*	DeVeaux *et al.* (1992)
GP584	*h*$^+$ *ade6-M26 swi5-134*	DeVeaux *et al.* (1992)
GP788	*h*$^+$ *ade6-M26 ura1-61*	L. DeVeaux[a]
GP814	*h*$^-$ *ade6-52 swi5-134 ura4-595*	J. Virgin[a]
GP1908	*h*$^-$ *ade6-M26 ura4-D18 lys4-95*	F. Li[a]
GP3652	*h*$^-$ *smt0 ade6-M26 rad50::kanMX pat1-114*	Young *et al.* (2004)
GP3999	*h*$^-$ *ade6-52 swi5-201::kanMX6*	Transformation[b] of GP13
GP4018	*h*$^+$ *ade6-52 swi5-201::kanMX6*	GP24 × GP3999
GP4019	*h*$^-$ *ade6-M26 swi5-201::kanMX6*	GP24 × GP3999
GP4020	*h*$^+$ *ade6-M26 swi5-201::kanMX6*	GP24 × GP3999
GP4075	*h*$^+$ *ade6-3049 lys3-37 pro1-1*	This study[a]
GP4165	*h*$^-$ *ade6-52 lys3-37 pro1-1*	GP289 × GP4075
GP4196	*h*^{90} *ade6-M26 swi5-134*	GP50 × GP584
GP4197	*h*^{90} *ade6-M26 swi5-201::kanMX6*	GP50 × GP4020
GP4213	*h*$^+$ *ade6-M26 swi5-201::kanMX6 ura1-61*	GP788 × GP4019
GP4216	*h*$^-$ *ade6-52 swi5-201::kanMX6 lys3-37 pro1-1*	GP4018 × GP4165
GP4367	*h*$^+$ *ade6-M26 swi5-134 ura4-D18*	GP584 × GP1908
HE415	*h*$^+$ *lys1 ade4-31*	Schmidt (1993)
HE416	*h*$^+$ *lys1 ade4-31 swi5-39*	Schmidt (1993)
HE424	*h*$^-$ *ura1-171 his6-365*	Schmidt (1993)
HE426	*h*$^-$ *ura1-171 his6-365 swi5-39*	Schmidt (1993)
HE596	*h*$^-$ *ade4-31 swi5::ura4*$^+$	This study
HE597	*h*$^+$ *lys3-131 swi5::ura4*$^+$	This study

×, meiotic cross.

[a] Genealogy available upon request.

[b] Transforming DNA was a PCR product using pFA6a-kanMX6 as template (Bähler *et al.* 1998). See materials and methods.

ing into the *Eco*RI site of plasmid pFY20 (Li *et al.* 1997) an *Eco*RI-digested product of a polymerase chain reaction (PCR). The PCR used DNA from strain GP3652 as template and primers 32 or 35 nucleotides long, with 5′-terminal *Eco*RI sites, designed to amplify the 1.2-kb region from bp 2699–3856 of cosmid SPBC409 (GenBank accession no. AL109822). Methods were as described in Ausubel *et al.* (2003). Plasmids were introduced into *S. pombe* cells by LiOAc-stimulated transformation as described by Ito *et al.* (1983).

Construction of *swi5-201::kanMX6*: The method of Bähler *et al.* (1998) was used. Primers 100 nucleotides long and plasmid pFA6A-kanMX6 as template were used in a PCR to generate a 1.6-kb DNA fragment with the *kanMX6* cassette flanked by 80 bp identical to the DNA immediately adjacent to the *swi5* gene (SPBC409.03; bp 3085–3164 and 3640–3719 on cosmid SPBC409). This fragment was purified by gel electrophoresis, extraction with a QIAquick kit (QIAGEN, Chatsworth, CA), and precipitation with ethanol. Approximately 1 μg was used to transform ~4 × 10^7 cells of strain GP13, with selection for G418 antibiotic resistance. Colonies were purified and tested for stability of resistance, and the mutation verified by nucleotide sequencing using primers ~200 bp outside the substituted region.

Culture media and meiotic crosses: Rich yeast extract media (liquid YEL and solid YEA), Edinburgh minimal media (liquid EMM2 and solid EMM2 agar), malt extract agar (MEA), and sporulation agar (SPA) are described by Gutz *et al.* (1974). Media were supplemented with adenine and other nutrients (100 μg/ml) as required. For meiotic crosses the two parental strains were grown to saturation in YEL and 100 μl (50 μl for Table 5) of each culture were mixed. The cells were collected by centrifugation, washed twice in 1 ml of H_2O, suspended in ~15 μl of H_2O, and deposited on SPA (MEA for Table 2). After 2 days at 25° spores were harvested and assayed for total viable spores and recombinants as described by Ponticelli and Smith (1989), except that EtOH treatment was for 10–15 min and Ade$^+$ recombinant frequencies were determined by plating on YEA with and without guanine (200 μg/ml). Crosses between strains GP814 and GP4367 (Table 3) were plated on EMM2 plus uracil with or without adenine; uracil was omitted for crosses involving a *ura4*$^+$ plasmid (pCE1, pCE2, or pFY20). For the experiments in Table 5 two colonies of each strain (*h*$^+$ or *h*$^-$) freshly grown on YEA + adenine were picked to separate tubes of YEL + adenine (5 ml) and grown to saturation; the four combinations of *h*$^+$ × *h*$^-$ matings, with control strains, were done and analyzed concurrently.

RESULTS

Cloning the *swi5* gene and sequencing *swi5* mutations: Using the iodine-positive reaction of *h*^{90} *swi*$^+$ colonies (Gutz *et al.* 1974), we obtained from an *S. pombe* genomic library

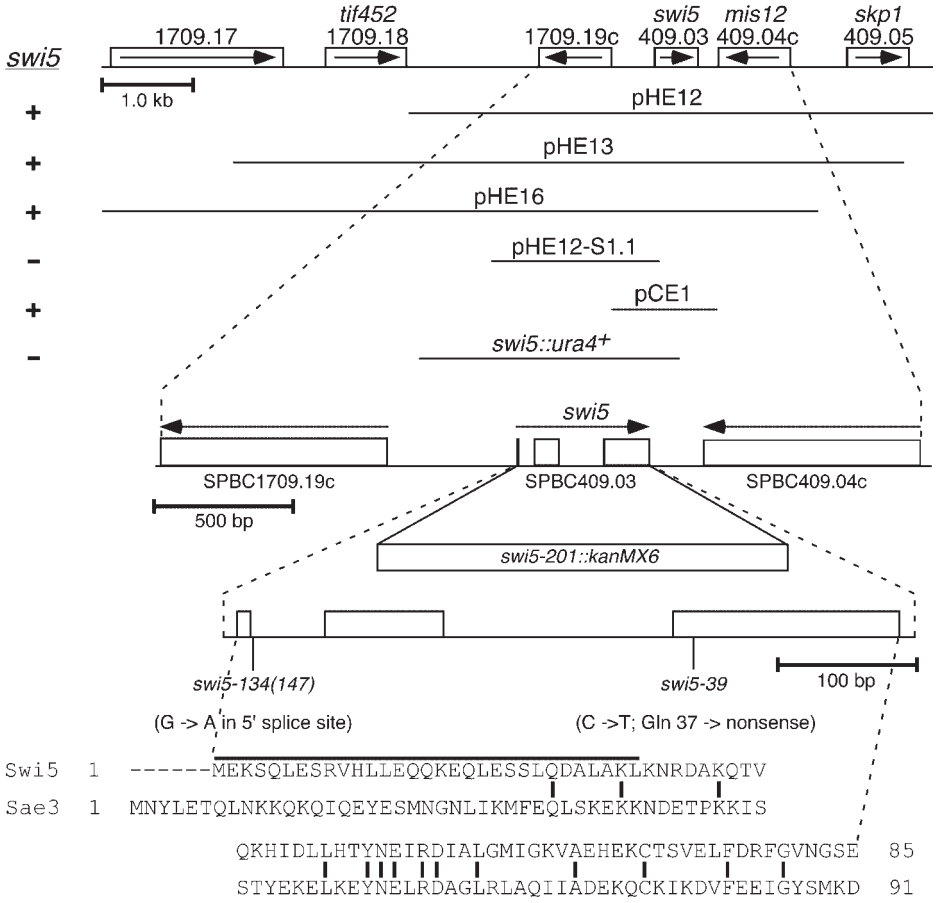

FIGURE 1.—Structure of the *swi5* gene and its surroundings on chromosome II of *S. pombe* and a comparison of Swi5 and *S. cerevisiae* Sae3. The top line shows a 9-kb segment of chromosome II contained on the overlapping cosmids SPBC1709 and SPBC 409; boxes with arrows indicate genes and their direction of transcription. *swi5* is identical to SPBC409.03 (see RESULTS). Lines below the top line indicate the extents of five clones and one deletion (*swi5::ura4*$^+$) and their ability to complement the *swi5-39* mutation (left column). The expanded line in the middle of the figure represents *swi5* and the two flanking genes. Open boxes indicate open reading frames (exons). Below this line are the *swi5-201::kanMX6* substitution and the positions of the *swi5* point mutations. At the bottom of the figure is a ClustalW alignment, with high gap penalty, of the deduced *S. pombe* Swi5 and *S. cerevisiae* Sae3 proteins (see DISCUSSION). Vertical bars indicate 14 identical amino acids, and the horizontal bar over the Swi5 sequence indicates a predicted coiled-coil structure (LUPAS 1996).

(FLECK *et al.* 1992) three independent clones that complemented the *swi5-39* mutation, initially characterized by GUTZ and SCHMIDT (1985; data not shown). Restriction digestion and nucleotide sequencing revealed that these clones, pHE12, pHE13, and pHE16, have at least three putative genes in common; the largest of these genes is SPBC1709.19c (Figure 1). Substitution of the 1.8-kb *Hin*dIII fragment containing *ura4*$^+$ (GRIMM *et al.* 1988) for 2.8 kb of SPBC1709.19c and its flanking DNA created an allele, designated *swi5::ura4*$^+$, that conferred UV sensitivity, mating-type switching deficiency, and meiotic recombination deficiency comparable to that of *swi5* mutants (Table 2 and data not shown). Unlike the previously described *swi5* mutants, however, strains with the *swi5::ura4*$^+$ allele grew slowly; furthermore, a subclone, designated pHE12-S1.1 and containing all of SPBC1709.19c (Figure 1), failed to complement *swi5-39* (data not shown). These results indicated that the *swi5* gene was on the initial clones but was not SPBC1709.19c.

On the basis of the information above, we determined the nucleotide sequence of the region flanking SPBC 1709.19c in the previously described *swi5* mutants. The *swi5-134* and *swi5-147* mutations (GUTZ and SCHMIDT 1985) were identical G → A changes 475 bp to the 5′ side of SPBC1709.19c, and *swi5-39* was C → T 795 bp to the 5′ side (Figure 1). We noted that these mutations were in a hypothetical small gene with two introns. A cDNA corresponding to part of the conceptual mRNA from this gene has been reported (GenBank accession no. AU013615; M. MORIMYO and K. MITO, unpublished data), supporting the notion that this hypothetical gene is active and that its RNA transcript is spliced as predicted. If so, the *swi5-134* and *147* mutations would change the beginning of the 5′ splice site of the first intron from the invariant 5′ GT . . . to 5′ AT . . ., and *swi5-39* would change codon 38 from CAA (Gln) to UAA (nonsense; Figure 1). The gene SPBC409.03, now designated *swi5*, is predicted to encode an 85-amino-acid polypeptide whose N-terminal ~30 amino acids are predicted to form a coiled-coil (LUPAS 1996). AKAMATSU *et al.* (2003) independently identified *swi5* as SPBC409.03.

Deletion of the *swi5* coding sequence and the null phenotype: On the basis of the above information, we deleted precisely the coding sequence of SPBC409.03 and replaced it with the *kanMX6* cassette to create the *swi5-201::kanMX6* allele, called here *swi5*Δ. Strains with this allele had a phenotype much like that of previously described *swi5* alleles. Mitotic growth was not significantly different from that of wild type (data not shown). Mating-type switching was much reduced and produced an iodine-staining reaction, indicative of mating and sporulation (GUTZ *et al.* 1974), comparable to that of *swi5-134* (data not shown). The deletion mutant was slightly more sensitive to UV irradiation than was *swi5-134*, but both mutants were

TABLE 2

***lys1-ade4* intergenic recombination in *swi5* mutants (tetrad analysis)**

h^- parent		h^+ parent					
HE no.	*swi5*	HE no.	*swi5*	PD	NPD	T	cM[a]
424	+	415	+	50[b]	55[b]	214[b]	~260
426	*39*	416	*39*	129[b]	9[b]	103[b]	35
426	*39*	416	*39*	37	4	50	51
597	Δ[c]	596	Δ	188	24	197	46

PD, parental ditype; NPD, nonparental ditype; T, tetratype.

[a] Genetic distance in centimorgans was calculated for the wild type from the physical distance (1.63 Mb) between *lys1* and *ade4* (Wood *et al.* 2002) and the genome mean of 0.16 cM/kb (Young *et al.* 2002) and for the *swi5* mutants using the equation of Haldane (1919).

[b] Data from Schmidt (1993).

[c] *swi5::ura4*$^+$.

considerably more sensitive than wild type (data not shown), as reported by Akamatsu *et al.* (2003).

To quantitatively evaluate complementation between *swi5* alleles, we measured meiotic recombination in *ade6* intragenic crosses (Table 3). Gene conversion at *ade6* was reduced slightly more by the *swi5*Δ substitution than by *swi5-134* (factors of 12 and 5, respectively, relative to wild type). These two alleles failed to complement, but two subclones, pCE1 and pCE2, containing the *swi5* coding sequence and all of the DNA between the two flanking genes (Figure 1), did complement *swi5-134* (Table 3). These results indicate that SPBC409.03 is *swi5*.

Crossing over was also reduced by a *swi5* deletion to about the same extent as by a *swi5* point mutation. By tetrad analysis, crossing over between *lys1* and *ade4* was reduced from ~260 cM in wild type to ~35–50 cM in *swi5* mutants, a reduction by a factor of ~6 (Table 2). By random spore analysis, recombination in the *lys3–ura1* and *ura1–pro1* intervals was reduced in the *swi5*Δ mutant by factors of 37 and 8, respectively (Table 4). The *swi5-39* mutation reduces crossing over in six additional intervals by factors of 4–17 (Schmidt 1993). In summary, *swi5* deletion mutants have phenotypes similar to those of the point mutants.

A *swi5* deletion suppresses the low viable spore yield of *mus81* mutants: The Mus81•Eme1 endonuclease appears to be a Holliday junction resolvase essential for meiotic crossing over (Boddy *et al.* 2001; Gaillard *et al.* 2003; Osman *et al.* 2003; Smith *et al.* 2003). *mus81* mutants have very low viable spore yields, presumably because joint molecules are formed but remain unresolved in the absence of this endonuclease. The low viable spore yield is suppressed by a *rec6* or *rec12* mutation, which blocks meiotic DNA breakage and presum-

TABLE 3

***ade6* intragenic recombination in *swi5* mutants**

ade6-52 parent			*ade6-M26* parent		Ade$^+$ recombinants per 10^6 viable spores[b]
GP no.[a]	*swi5*	Plasmid	GP no.	swi5	
13	+	—	24	+	3500 ± 500
3999	*swi5* Δ[c]	—	24	+	2700 ± 430
583	*swi5-134*	—	584	*swi5-134*	680 ± 80
3999	*swi5* Δ	—	584	*swi5-134*	520 ± 80
3999	*swi5* Δ	—	4020	*swi5* Δ	280 ± 30
814	*swi5-134*	—	4367	*swi5-134*	50 ± 13[d]
814	*swi5-134*	pCE1[e]	4367	*swi5-134*	3400 ± 650[f]
814	*swi5-134*	pCE2[e]	4367	*swi5-134*	4000 ± 860[f]
814	*swi5-134*	pFY20	4367	*swi5-134*	530 ± 120[f]

—, no plasmid present.

[a] For plasmid-containing strains, the GP number indicates the parent strain without a plasmid.

[b] The recombinant frequencies in three independent matings are given as the mean ± SEM.

[c] *swi5-201::kanMX6.*

[d] The lower frequency in these crosses than in crosses between GP583 and GP584 appears to stem, for unknown reasons, from the *ura4* mutation in strains GP814 and GP4367 (data not shown).

[e] Contains a 1.2-kb *swi5* fragment in opposite orientations in the vector pFY20 (see materials and methods).

[f] The recombinant frequencies of two experiments with two independent transformants are given ($n = 4$).

TABLE 4

Recombination in *swi5* mutants (random spore analysis)

h^- parent[a]		h^+ parent[a]		% recombinants[b]		Ade$^+$ recombinants/ 10^6 viable spores
GP no.	*swi5*	GP no.	*swi5*	*lys3–ura1*	*ura1–pro1*	
4165	+	788	+	14.7	27.6	4400
4216	Δ[c]	4213	Δ	0.4	3.4	320

[a] The h^- parent was *ade6-52 lys3-37 pro1-1*; the h^+ parent was *ade6-M26 ura1-61*.
[b] From each cross 232 segregants were tested; reciprocal types were nearly equally frequent.
[c] *swi5-201::kanMX6*.

ably joint molecule formation (Cervantes *et al.* 2000; Boddy *et al.* 2001; Young *et al.* 2002; Osman *et al.* 2003). Because *swi5* mutants, like *rec6* and *rec12* mutants, are deficient in meiotic recombination but have relatively high viable spore yields (Schmidt *et al.* 1987; Ponticelli and Smith 1989; DeVeaux *et al.* 1992; Schmidt 1993; Lin and Smith 1994; Young *et al.* 2004), we tested the ability of a *swi5*Δ mutation to suppress the low viable spore yield of a *mus81* mutation. The *mus81*Δ mutation decreased the viable spore yield, relative to that of wild type, by a factor of >10,000; this low level was increased >700-fold by *swi5*Δ (Table 5). Although this suppression was strong, it was not complete, as the viable spore yield was lower than that of *swi5*Δ by a factor of ~6. These results suggest that Swi5 and perhaps other factors act before Mus81•Eme1. The Ade$^+$ recombinant frequency was indistinguishable in *swi5*Δ and *swi5*Δ *mus81*Δ strains, indicating that Mus81•Eme1 has little effect on intragenic recombination (gene conversion) in *swi5*Δ mutants, as in *swi5*$^+$ strains (Osman *et al.* 2003; Smith *et al.* 2003).

Viable spore yields are reduced synergistically by *swi5* and an *rhp55* or *rhp57* mutation but not a *dmc1* mutation: The accumulation of broken DNA in a *swi5* mutant (Young *et al.* 2004) and the suppression of *mus81*Δ by *swi5*Δ (Table 5) suggest that Swi5 acts during the DNA strand-exchange step of recombination. Other proteins have been inferred to act at this step; these proteins include the DNA strand-exchange factors Rad51 and Dmc1 and the accessory factors Rhp55 and Rhp57 (Grishchuk and Kohli 2003 and references therein). This inference suggests that there might be genetic interactions among mutations in the genes encoding these proteins. To test this possibility, we measured meiotic recombination and viable spore yields in single and double mutants.

We first tested interaction of *swi5*Δ and *dmc1*Δ. Intragenic *ade6* recombination was reduced by a factor of ~2 by *dmc1*Δ and by a factor of ~14 by *swi5*Δ (Table 5). In the *swi5*Δ *dmc1*Δ double mutant, recombination was intermediate between the levels in the single mutants. Viable spore yields were only modestly reduced, by a factor of ~2 or less in these mutants, but again

TABLE 5

swi5* interacts with *mus81* and oppositely with *rhp55* and *rhp57

		swi5$^+$		*swi5*Δ	
Mutant[a]	n[b]	Ade$^+$/10^6 viable spores[c]	Viable spores/cell[c]	Ade$^+$/10^6 viable spores[c]	Viable spores/cell[c]
+	12	3700 ± 210	≡100	270 ± 80	44
*mus81*Δ	8	ND	<0.01	250 ± 52	6.8
*mus81*Δ *dmc1*Δ	4	1200 ± 470	0.25	400 ± 23	6.5
*dmc1*Δ	8	1600 ± 95	81	470 ± 45	58
*rhp55*Δ	4	2100 ± 280	33	ND	<0.1
*rhp57*Δ	4	1200 ± 130	11	ND	<0.001

ND, not determined, as too few viable spores were produced for accurate measurement.
[a] Alleles were *mus81::kanMX6* (Boddy *et al.* 2001), *dmc1::ura4*$^+$ (Fukushima *et al.* 2000), *rhp55::ura4*$^+$ (Khasanov *et al.* 1999), *rhp57::ura4*$^+$ (Tsutsui *et al.* 2000), and *swi5-201::kanMX6* (materials and methods) and were homozygous mutant or wild type as indicated. One parent was *ade6-M26*, and the other *ade6-52*. Strains with *rhp55*Δ or *rhp57*Δ were h^+ or h^- *smt-0* (Styrkarsdottir *et al.* 1993), and those with *rhp55*Δ, *rhp57*Δ or *dmc1*Δ were *ura4-D18* (Grimm *et al.* 1988). Complete genotypes and genealogies are available upon request.
[b] Number of independent matings analyzed for *swi5*$^+$ and *swi5*Δ. Matings were in sets of four for each genotype, done on three different days for $n = 12$ and on two different days for $n = 8$ (see materials and methods).
[c] Mating and meiosis were on SPA at 25°. Ade$^+$ recombinant frequencies are the means ± SEM. Viable spore yields are expressed relative to wild-type matings, which produced 8.5 ± 0.96 ($n = 12$) viable spores per viable cell (of the less numerous parent) added to the mating mixture. This yield is greater than the theoretical four spores per limiting haploid cell because of slight residual growth of cells on the sporulation medium.

the phenotype of the double *swi5*Δ *dmc1*Δ mutant was intermediate between those of the single mutants.

The similar phenotypes of *swi5*Δ, *dmc1*Δ, and *swi5*Δ *dmc1*Δ mutants suggest that Swi5 and Dmc1 act at closely related steps of meiosis. We therefore tested interaction of *dmc1*Δ with *mus81*Δ. Like *swi5*Δ, the *dmc1*Δ mutation increased the viable spore yield of *mus81*Δ, by a factor of >25 but below the level of *mus81*Δ *swi5*Δ. The *ade6* recombinant frequency in the *dmc1*Δ *mus81*Δ mutant was near that of the *dmc1*Δ single mutant (Table 5). The triple mutant *mus81*Δ *swi5*Δ *dmc1*Δ had a phenotype similar to that of the *mus81*Δ *swi5*Δ double mutant. These results support the suggestion that Swi5 and Dmc1 act at closely related steps before Mus81•Eme1.

In contrast to the *swi5*Δ *dmc1*Δ double mutant, which had a phenotype similar to that of the single mutants, double mutants with *swi5*Δ and either *rhp55*Δ or *rhp57*Δ had a much stronger phenotype than the single mutants. Viable spore yields were reduced, relative to that of wild type, by factors of ~2–10 in the three single mutants (Table 5). The addition of the *swi5*Δ mutation to the *rhp55*Δ or *rhp57*Δ mutant reduced the viable spore yields by factors of >300 and >10,000, respectively. These yields were too low to allow a reliable measure of recombination. These results indicate that Swi5 acts at a step different from that of Rhp55 and Rhp57.

In summary, the *swi5*Δ mutation interacted strongly with *mus81*Δ, *rhp55*Δ, and *rhp57*Δ mutations, but only weakly with *dmc1*Δ. *dmc1*Δ also interacted with *mus81*Δ, but less strongly than did *swi5*Δ. Below, we discuss interpretations of these results.

DISCUSSION

Swi5 is a remarkably small protein—85 amino acids—that appears, from the evidence discussed below, to act with other proteins to form joint DNA molecules. This activity is important in the repair of single- and double-strand DNA breaks during both mitotic growth and meiosis.

Swi5 is related to the small Sae3 protein of *S. cerevisiae* (Figure 1). *sae3* mutants were identified among sporulation-deficient mutants that are rescued by a *spo11* mutation, a phenotype consistent with Sae3 acting after DNA break formation by Spo11 (McKee and Kleckner 1997). *SAE3* was thought to encode a 50-amino-acid polypeptide from a single exon (McKee and Kleckner 1997), but further analysis suggests an additional exon and a protein of 91 amino acids (A. Shinohara, personal communication). This longer version has 14 amino acids identical to those of Swi5 scattered throughout the proteins (Figure 1). Physical analyses show that Sae3 and Swi5 are required for the repair of meiotic DNA double-strand breaks (McKee and Kleckner 1997; Young *et al.* 2004) and appear to act in concert with RecA-like strand exchange proteins. *S. cerevisiae sae3* mutants have a phenotype indistinguishable from that of *dmc1* mutants: both are meiosis specific, accumulate broken DNA hyperresected at the ends, and form less recombinant DNA than wild type does; the *sae3 dmc1* double mutant has the same phenotype as the single mutants (McKee and Kleckner 1997). These results suggest that Sae3 and Dmc1, a meiosis-specific DNA strand-exchange protein (Bishop *et al.* 1992; Sehorn *et al.* 2004), act in the same step in DNA repair, the formation of joint molecules.

There are differences in the roles of Swi5 and Sae3, however. Although Sae3 is meiosis specific, Swi5 is required during mitotic growth for repair of DNA damage (Schmidt *et al.* 1989; Akamatsu *et al.* 2003; data not shown) and for mating-type switching (Egel *et al.* 1984). In addition, although the phenotypes of *S. cerevisiae sae3* and *dmc1* mutants are indistinguishable, the phenotypes of *S. pombe swi5* and *dmc1* mutants differ: during meiosis broken DNA accumulates in *swi5* mutants but not in *dmc1* mutants (Young *et al.* 2004), spore viabilities are lower in *swi5* mutants than in *dmc1* mutants (Grishchuk and Kohli 2003; Young *et al.* 2004; Table 5), and intragenic recombinant frequencies are lower in *swi5* mutants than in *dmc1* mutants (Schmidt *et al.* 1987; DeVeaux *et al.* 1992; Schmidt 1993; Fukushima *et al.* 2000; Grishchuk and Kohli 2003; Table 5). Thus, *S. pombe* Swi5 and Dmc1 are not functionally identical, although *S. cerevisiae* Sae3 and Dmc1 appear to be. Below, we discuss the roles of these and related proteins in meiotic and mitotic DNA break repair.

Meiotic DNA break repair: During meiosis in *S. pombe* ds DNA breaks are made at high frequency at widely separated sites in the genome (Cervantes *et al.* 2000; Young *et al.* 2002). Formation of these breaks requires Rec12, a homolog of *S. cerevisiae* Spo11, which contains the active site for breakage (Keeney *et al.* 1997); at least nine additional proteins are also needed for high-frequency breakage (Cervantes *et al.* 2000; Young *et al.* 2002, 2004). Repair of these breaks requires several identified proteins, including Rad32, Rad50, Swi5, and Rad51 (Young *et al.* 2004). Rad32•Rad50•Nbs1 is a homolog of the *S. cerevisiae* Mre11•Rad50•Xrs2 complex, which is required to remove Spo11 covalently linked to the 5′ end of the broken DNA and to form ss DNA tails (Haber 1998a). Rad51 is a homolog of the *S. cerevisiae* Rad51 protein, which, like *Escherichia coli* RecA, promotes homologous DNA strand exchange between ss and ds DNA to form joint molecules (Sung *et al.* 2000). Optimal activity of Rad51, however, requires additional proteins, such as Rad54, Rad55, and Rad57. Rad55 and Rad57 form a tight heterodimeric complex and share limited amino acid sequence identity with Rad51; the Rad55•Rad57 complex may aid Rad51 by forming a complex with Rad51 (Sung *et al.* 2000). In *S. pombe,* a *rad51* null mutation has a stronger meiotic phenotype than those of *rhp55*, *rhp57*, or *dmc1* with respect to spore viability and recombinant frequencies (Grishchuk and Kohli 2003). Double-mutant analyses suggest that Rhp55 and Rhp57 act on one branch of a DNA repair pathway and

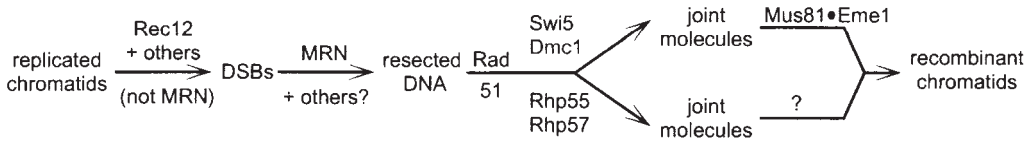

FIGURE 2.—Proposed branched pathway for meiotic recombination in *S. pombe*. After meiotic DNA replication, Rec12, aided by other proteins, makes DNA breaks independently of Rad32•Rad50•Nbs1 (the MRN complex; CERVANTES *et al.* 2000; YOUNG *et al.* 2004). The MRN complex, perhaps in conjunction with other proteins, resects one DNA strand. The resultant ss DNA forms a joint molecule with an intact homolog by either of two branches—one promoted by Swi5 plus Dmc1 and the other by Rhp55 plus Rhp57—each of which may require Rad51. The Swi5-Dmc1-dependent joint molecules are resolved by Mus81•Eme1 into recombinant chromatids (BODDY *et al.* 2001; OSMAN *et al.* 2003; SMITH *et al.* 2003). Functions required for joint molecule resolution by the Rhp55-Rhp57-dependent branch have not been determined. This pathway is similar to one proposed by GRISHCHUK and KOHLI (2003) and relies in part upon observations of meiotic recombination in *S. cerevisiae* (ROEDER 1997).

Dmc1 on another; both of these branches are proposed to require Rad51 (GRISHCHUK and KOHLI 2003).

Our results (Table 5) support the proposal of a pathway of meiotic DNA break repair with two branches leading to joint molecules (Figure 2). From the similar phenotypes of *swi5*Δ, *dmc1*Δ, and *swi5*Δ *dmc1*Δ mutants we infer that Swi5 and Dmc1 act in one branch of the pathway. From the strong synergisms between *swi5*Δ and *rhp55*Δ and between *swi5*Δ and *rhp57*Δ, we infer that Rhp55 and Rhp57 act in another branch. The Mus81•Eme1 endonuclease is required to resolve joint molecules, presumably made by Rad51, Dmc1, Swi5, Rhp55, and Rhp57, into crossover recombinants; in *mus81*Δ mutants very few viable spores are produced (BODDY *et al.* 2001; GAILLARD *et al.* 2003; OSMAN *et al.* 2003: SMITH *et al.* 2003; Table 5). Since *swi5*Δ and *dmc1*Δ suppress the low viable spore yield of *mus81*Δ mutants, we infer that Swi5 and Dmc1 act before Mus81. These inferences are concordant with current knowledge of the enzymatic activities of the proteins and indicate that Swi5 and Dmc1, and perhaps Rhp55 and Rhp57 as well, lead to joint molecules that are resolved by Mus81•Eme1.

Mitotic DNA break repair and mating-type switching: During mitotic growth, the recovery from DNA damage is aided by several proteins. Mutant analyses suggest that Rad51, Rhp55, Rhp57, and Swi5 have relationships similar to those in meiosis. For example, *rhp57* and *swi5* mutants are mildly sensitive to UV and gamma irradiation, but the double mutant is as sensitive as *rad51* or *rad51 swi5* (AKAMATSU *et al.* 2003; data not shown). Although the nature of the UV- and gamma-ray-induced lesions is not certain, these lesions may be primarily ss and ds breaks, respectively; if so, Swi5, as well as Rhp57 and perhaps Rhp55, would seem to aid Rad51 in the repair of both types of breaks.

Mating-type switching in mitotic *S. pombe* cells requires Swi5 and is initiated by a ss lesion at the *mat1* locus; this lesion may be converted into a ds break during replication (ARCANGIOLI and THON 2003; KAYKOV *et al.* 2004). Single-strand DNA with a 3′ end at the *mat1* lesion presumably undergoes strand exchange with homologous DNA flanking the *mat2* or *mat3* locus; DNA synthesis primed by this end produces a copy of *mat2* or *mat3*, which replaces the copy at *mat1* (ARCANGIOLI and DE LAHONDES 2000). Thus, mating-type switching is a type of copy-choice recombination, which relies on joint molecule formation by DNA strand transfer, as do break-join and break-copy recombination. *swi5* mutants have normal levels of the *mat1* lesion but rarely switch mating type. The inability of *swi5* mutants to use the lesion to promote switching is consistent with the Swi5 protein being required for high-level DNA strand exchange, *i.e.*, joint molecule formation. The mechanism of resolution of these joint molecules is unclear, since mating-type switching is not notably affected by *mus81*Δ (our unpublished observations). Nevertheless, we infer that the primary function of Swi5 is the same in meiotic and mitotic cells—the formation of joint molecules during DNA break repair.

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