swi6, a Gene Required for Mating-Type Switching, Prohibits Meiotic Recombination in the *mat2-mat3* "Cold Spot" of Fission Yeast

Amar J. S. Klar and Michael J. Bonaduce

NCI-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Laboratory of Eukaryotic Gene Expression, Frederick, Maryland 21702–1201

Manuscript received March 18, 1991
Accepted for publication August 12, 1991

ABSTRACT

Mitotic interconversion of the mating-type locus (*mat1*) of the fission yeast *Schizosaccharomyces pombe* is initiated by a double-strand break at *mat1*. The *mat2* and *mat3* loci act as nonrandom donors of genetic information for *mat1* switching such that switches occur primarily (or only) to the opposite *mat1* allele. Location of the *mat1* "hot spot" for transposition should be contrasted with the "cold spot" of meiotic recombination located within the adjoining *mat2-mat3* interval. That is, meiotic interchromosomal recombination in *mat2, mat3* and the intervening 15-kilobase region does not occur at all. *swi2* and *swi6* switching-deficient mutants possess the normal level of double-strand break at *mat1*, yet they fail to switch efficiently. By testing for meiotic recombination in the cold spot, we found the usual lack of recombination in a *swi2* mutant but a significant level of recombination in a *swi6* mutant. Therefore, the *swi6* gene function is required to keep the donor loci inert for interchromosomal recombination. This finding, combined with the additional result that switching primarily occurs intrachromosomally, suggests that the donor loci are made accessible for switching by folding them onto *mat1*, thus causing the cold spot of recombination.

MEIOTIC recombination events are not uniformly distributed along the chromosome; rather, there are specific sites called "hot spots" which act as localized stimulators of genetic exchange (reviewed by SZOSTAK *et al.* 1983), or "cold spots," which exhibit depressed levels of recombination. Although a large number of studies have been conducted on recombination hot spots, only a few studies have addressed the reason for cold spots. One such cold spot exists between the *mat2* and *mat3* loci in *Schizosaccharomyces pombe*. These loci contain unexpressed *P*lus (*P*) and *M*inus (*M*) "cassettes," a copy of which is transposed to the *mat1* locus where that information is expressed. This process causes a switch of the mating-type locus (Figure 1). The *mat2-P* locus is situated 15 kilobases (kb) distal to *mat1* and contains the *P* information, while *mat3* is 15 kb distal to *mat2* and contains the *M* information. The *his2* marker is located 1 centimorgan (cM, meiotic distance) distal to *mat2* (LEUPOLD 1958). The interval between *mat2* and *mat3* is called the K region (Figure 1). Genetically, *mat1* and *mat2* are 1 cM apart (LEUPOLD 1958), while *mat2* and *mat3* segregate as one locus, since no meiotic recombinants between *mat2* and *mat3* were observed in 17,000 segregants selected to be recombinants for the flanking *his7* and *his2* markers (EGEL 1984). Therefore, *mat2* and *mat3* are separated genetically by a map distance of less than 0.001 cM. Based on the overall genetic map length of *S. pombe*, the 15-kb intervening sequence should place the *mat2* and *mat3* loci about 3 cM apart. These two loci were shown to be distinct by molecular studies (BEACH 1983; BEACH and KLAR 1984).

mat1 switching occurs efficiently during mitotic growth, as often as every generation, where yeast cells alternate between *P* (*mat1-P*) and *M* (*mat1-M*) cell types (LEUPOLD 1950; EGEL 1977; MIYATA and MIYATA 1981; EGEL and EIE 1987; KLAR 1990). The mating-type switching is initiated by a site-specific double-stranded break (DSB) at the *mat1* locus (BEACH 1983; BEACH and KLAR 1984; EGEL, BEACH and KLAR 1984; KLAR and MIGLIO 1986; KLAR 1987; NIELSEN and EGEL 1989; KLAR, BONADUCE and CAFFERKEY 1991). The pattern of *mat1* switching is highly programmed, such that only one among four granddaughters of a cell ever switches to the opposite mating type in about 90% of the pedigrees (MIYATA and MIYATA 1981), and the sister of the recently switched cell switches efficiently in consecutive cell divisions (EGEL and EIE 1987; KLAR 1990). Both of these so-called "rules of switching" are now believed to be due to inheritance of specific parental and grandparental DNA chains by progeny cells (KLAR 1987, 1990).

As a switching-competent cell switches to the opposite allele in about 90% of cell divisions, this feature implies a nonrandom choice of the donor during each switch. The reason that cells switch primarily or only to the opposite allele is not understood. It is possible that the meiotic recombination cold spot between *mat2* and *mat3* is one manifestation of the special

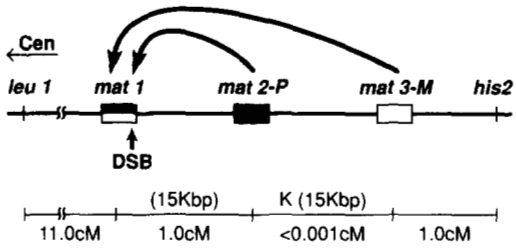

FIGURE 1.—The *mat* region on chromosome *II*, showing *mat1*, *mat2-P* and *mat3-M* cassettes. The centromere (Cen) is located about 65 cM to the left of *mat1*. Arrows indicate that *mat1* switches occur by unidirectional transfer of DNA from *mat2* and *mat3* loci into *mat1*. DSB marks the site of a double-stranded break at *mat1* that efficiently initiates recombination. Switching involves unidirectional transfer of a ~1.1-kb allele-specific sequence copied from *mat2* or *mat3* to *mat1* by a gene conversion event. The 59-bp homology sequence (H1) on one side and the 135-bp homology sequence (H2) on the other side of the allele-specific sequence, present in all three cassettes, are probably used as pairing sites for the gene-conversion event. The interval between *mat1* and *mat2* is 15 kb and the interval between *mat2* and *mat3*, called the K region, is also 15 kb. Physical distances between *leu1* and *mat1* and between *mat3* and *his2* are not known. Meiotic map distances (in cM) of markers flanking the *mat* region are also shown. The figure is derived from data presented earlier (LEUPOLD 1958; EGEL and GUTZ 1981; BEACH 1983; EGEL 1984; EGEL, BEACH and KLAR 1984; BEACH and KLAR 1984; KELLY *et al.* 1988).

organization of the mating-type region in the nucleus. It may be organized in a higher order chromatin structure whereby *mat2* and *mat3* are made readily accessible to *mat1* for recombination; for instance, by intrachromosomal folding them onto *mat1*. Furthermore, we may imagine that in the *mat1-M* cell the *mat2-P* donor is more accessible for recombination and, conversely, in the *mat1-P* cell the *mat3-M* donor is more accessible, resulting in the observed directionality of switching. Thus, by such a model, the *mat2*, *mat3* and K regions are folded onto *mat1* and consequently are precluded from recombination between the homologs causing the cold spot of recombination. For recent reviews of the system, refer to EGEL (1989) and KLAR (1989).

A curious mutant class of switch genes, *swi2*, *swi5* and *swi6*, was shown to possess the normal level of the DSB but switch inefficiently. It was speculated that these mutants are defective in utilization of the DSB at *mat1* for mating-type switching (EGEL, BEACH and KLAR 1984). Among these, the *swi5* gene was shown to be required for general recombination; thus, this function is not specific for mating-type switching. General recombination is not affected in *swi2* and *swi6* mutants, thus these genes are suggested to be specifically required for *mat1* switching (SCHMIDT, KAPITZA and GUTZ 1987). We reasoned that the *swi2* and *swi6* gene functions could be involved in utilization of the DSB by affecting the hypothesized donor accessibility. We have addressed this hypothesis by testing the effect of mutations on meiotic recombination within and between the *mat2* and *mat3* loci in *swi2*$^-$ and *swi6*$^-$ strains. Gene conversions and crossover events between the *mat2* and *mat3* loci were found in *swi6* mutants but not in *swi2* mutants.

MATERIALS AND METHODS

Strains: All *Schizosaccharomyces pombe* strains and their genotypes are presented in Table 1. All of the strains were constructed in this laboratory.

***mat* alleles defined:** Although important features of various *mat* alleles have been described in the text, we collectively define them here for a quick reference. The wild-type *mat* region containing *mat1*, *mat2* and *mat3* loci is designated h^{90} (*h* for *h*omothallism, *i.e.*, capable of switching mating type so as to produce 90% asci in colonies). *mat1-P* and *mat1-M* are alternate alleles of the mating-type locus *mat1*. *mat1-smt-s* is a *cis*-acting small deletion located distal to *mat1* that reduces the efficiency of switching by lowering the level of the DSB. *mat1-M-smt-o* and *mat1-PΔ17* are slightly larger *cis*-acting deletions that lack the DSB and thus are switching-deficient; these mutations do not otherwise affect the mating and sporulation functions of these alleles. The *mat2:3-M* allele is a fusion between *mat2* and *mat3* cassettes associated with deletion of the K region and the *P* cassette. *mat2,3Δ::LEU2* is a deletion of *mat2*, *mat3* and the K region into which the *Saccharomyces cerevisiae LEU2* gene has been placed. The *LEU2* gene weakly complements a mutation in the *leu1* gene of *S. pombe* (BEACH and NURSE 1981). Following the segregation of *leu1* marker in meiotic crosses involving strains containing *mat2,3Δ::LEU2* was unambiguous since *leu1*$^+$ *mat2,3Δ::LEU2* segregants grew to confluence within 24 hr in *leu*$^-$ medium, while those with *leu1*$^-$ *mat2,3Δ::LEU2* genotype required 48 hr for confluent growth. The *mat1Δ-1* is a 7.7 kb deletion removing sequences from the *mat1*-proximal *Xho*I site to the *mat1*-distal *Pvu*II site (BEACH 1983). Its construction will be described elsewhere. As the deletion-containing strain is sterile, it was crossed to other strains by the protoplast fusion technique (VAN SOLINGEN and VAN DER PLATT 1977).

Construction of the *mat2,3Δ::leu2*$^-$ allele: This allele was constructed by filling in the *Bst*EII site in the *LEU2* coding region of *mat2,3Δ::LEU2* construct described previously (KLAR and MIGLIO 1986) with DNA polymerase I Klenow fragment and then ligating the blunt ends with T4 DNA ligase. The construct was made *in vitro* and then placed into the genome by homologous recombination following the procedures described by MORENO, KLAR and NURSE (1991). Filling in the *Bst*EII site produced a frame-shift mutation in *LEU2*.

Culture conditions: We used standard conditions for culture, sporulation, tetrad and random spore analyses and construction of diploids from haploid cells (GUTZ *et al.* 1974; MORENO, KLAR and NURSE 1991).

Pedigree analysis: The switching efficiency was determined by the pedigree analysis of single cells according to MIYATA and MIYATA (1981). The procedure consists of determining the mating efficiency of progeny cells with each other during clonal growth. The resulting zygotic cells were observed microscopically. Switching occurs in a highly regulated fashion such that only one in four granddaughters of cell ever switches (MIYATA and MIYATA 1981).

Identification of mutations in *mat2* and *mat3* loci: The alleles at *mat2* and *mat3* were identified by mating the strain in question with cells from *mat1-P mat2,3Δ::LEU2* and *mat1-M mat2,3Δ::LEU2* testers. The h^{90} *mat2-P-B102* strain consists of a mixture of *mat1-M* and *mat1-P-B102*-containing cells. This strain would sporulate after mating with the *mat1-*

TABLE 1

Experimental strains of *S. pombe*

Strain	*mat* region	Genotype
PB30	*mat1-PΔ17*	*leu1-32 ura4 ade6-M210*
SP1001	*mat1-M-smt-o*	*his2 ade6-M216*
SP473	*mat1-P-smt-s mat2:3-M*	*leu1-32*
SP465	*mat1-M mat2:3-M*	*his2 leu1-32 ade6-M216*
SP452	*mat1-P-smt-s mat2,3Δ::LEU2*	*leu1-32 his2 ade6-M216*
SP6	*mat1-M mat2:3-M*	*leu1-32*
SP804	*mat1-P mat2,3Δ::leu2⁻*	*leu1-32 ade6-M216 ura4*
SP453	*mat1-M-smt-s mat2,3Δ::LEU2*	*leu1-32 his2 ade6-M210*
SP905	*mat1Δ-1/mat1-Por M mat2,3Δ::leu2⁻*	*his2/+ +/ura4 ade6-M216/ade6-M210*
SP1054	*h⁹⁰/mat1Δ-1 mat2,3Δ::leu2⁻*	*leu1-32/leu1-32 his2/+ ade6-M216/ade6-M216 +/ura*
SP975	*mat1-P mat2,3Δ::LEU2*	*swi2-73 ade6-M210*
SP956	*mat1-M mat2,3Δ::LEU2*	*swi2-73 leu1-32 his2 ade6-M216*
SP962	*mat1-P mat2,3Δ::LEU2*	*swi6-115 leu1-32 his2 ade6-M216*
SP960	*mat1-M mat2,3Δ::LEU2*	*swi6-115 ade6-M210*
SP914	*h⁹⁰ (mat3-M-B-406)*	*swi2-73 his2 ade6-M210 lys1*
SP909	*h⁹⁰ (mat2-P-B102)*	*swi2-73 leu1-32 ade6-M216*
SP948	*h⁹⁰ (mat3-M-B406)*	*swi6-115 leu1-32 ade6-M210*
SP965	*h⁹⁰ (mat2-P-B102)*	*swi6-115 his2*
SP908	*h⁹⁰ (mat3-M-B406)*	*swi6-115 leu1-32 ade6-M210*
SP916	*h⁹⁰ (mat2-P-B406)*	*swi6-115 his2*
SP876	*mat1-P mat2,3Δ::LEU2*	*swi6-115 leu1-32 ade6-M210*
SP926	*h⁹⁰*	*swi6-115 his2 ade6-M216*
SP1002	*h⁹⁰/mat1-M-smt-o*	*+/leu1-32 his2/+ ade6-M216/ade6-M210*
SP1003	*h⁹⁰/mat1-M-smt-o*	*swi2-73/swi2-73 +/leu1-32 his2/+ ade6-M216/ade6-M210 +/lys1*
SP1004	*h⁹⁰/mat1-M-smt-o*	*swi6-115/swi6-115 +/leu1-32 his2/+ ade6 M216/ade6-M210*
SP107	*h⁹⁰*	*swi6-115 his2 ade6-M210*
SP997	*mat1-PΔ17*	*swi6-115 leu1-32 ura4 ade6-216*
SP931	*mat1-M-smt-o*	*swi6-115 leu1-32 ade6-M216*
SP998	*mat1-PΔ17*	*swi6-115 leu1-32 his2 ade6-M210*

P tester but not with the *mat1-M* tester. Likewise, the *h⁹⁰ mat3-M-B406* strain would sporulate after mating to *mat1-M* but not to the *mat1-P* tester, and the *h⁹⁰ mat2-P-B102 mat3-M-B406* recombinant strains would not sporulate by mating with either tester.

RESULTS

The cold spot reduces recombination both to the left and to the right of the *mat2-mat3* interval: It was previously shown that the map distance of 2.0 cM found between *mat1* and *his2* markers in standard strains (LEUPOLD 1958) paradoxically expands to 12.0–18.0 cM in strains in which the chromosome is made shorter by about a 17-kb deletion of *mat2*, *mat3* and the K region (KLAR and MIGLIO 1986). The interval on the left between *leu1* and *mat1* remains unchanged in donor-deleted strains. In such crosses, the *mat2,3Δ::LEU2* allele, consisting of the *mat2*, *mat3*, and internal K-region deletion substituted with the *S. cerevisiae LEU2* gene, was employed. In these strains a normal level of the DSB is found at *mat1*, and cells must be able to repair the DSB even without switching (as they lack the donor loci) since deletion does not cause lethality.

The 2.0-cM map distance observed between *mat1* and *his2* markers in an earlier study (LEUPOLD 1958) was based on crosses with heterothallic strains. One strain contained *mat2*, K and *mat3* at *mat1* (the *h⁺ᴺ* rearrangement) while the other contained a single *mat2:3-M* donor resulting from fusion between *mat2* and *mat3* (BEACH and KLAR 1984). In principle, such rearrangements may alter the length of the *mat1-his2* interval. All the studies of this paper do not involve such rearrangements. Thus, as a base line control we determined the *mat1* to *his2* distance in a cross between strains PB30 (*mat1-PΔ17*, see Table 1 for complete genotype) and SP1001 (*mat11-M-smt-o*), both of which do not carry such rearrangements. The *mat1-PΔ17* allele consists of a small *mat1*-distal *cis*-acting deletion that is totally deficient in switching, as essential *cis*-acting elements required for generating the DSB have been deleted (ARCANGIOLI and KLAR 1991). The tight switching-deficient *cis*-acting *smt-o* mutation (ENGELKE *et al.* 1987) is likewise a small deletion of the *mat1*-distal sequences (O. NIELSEN and R. EGEL, personal communication). The strains were mated and zygotic asci were analyzed. As shown in Table 2, we observed a 0.5 cM distance between *mat1* and *his2* markers, a value significantly lower than the 2.0 cM reported earlier in a study involving strains containing rearrangements in the *mat* region (LEUPOLD 1958).

To determine whether deletion of the K region alone is sufficient to remove the cold spot, we per-

TABLE 2

Effect of *mat2*, *mat3* or K-region deletions on *mat1-his2* genetic length

Cross	Genotype	Map distance (cM)[a] *mat1-his2*	Increase in *mat1-his2* interval
PB30 × SP1001	*mat1-PΔ17* / *mat1-M-smt-o*	0.5 (439:0:4)[b]	Control
SP473 × SP465	*mat1-P-smt-s mat2:3-M* / *mat1-M mat2:3-M*	5.9 (227:2:17)	12-fold[c]
SP452 × SP6	*mat1-P-smt-s mat2,3Δ::LEU2* / *mat1-M mat2:3-M*	8.0 (203:1:32)	16-fold
SP804 × SP453	*mat1-P mat2,3Δ::leu2⁻* / *mat1-M-smt-s mat2,3Δ::LEU2*	10.8 (116:0:32)	22-fold

The *mat* region mutations and deletions are defined in the text.

[a] Map distance in cM was calculated with the equation cM = 100 [(TT + 6NPD)/2 (PD + NPD + TT)] according to PERKINS (1949). PD, parental ditype; NPD, nonparental ditype; TT, tetra type.

[b] The numbers in brackets represent observed frequency of PD:NPD:TT tetrads observed for each pair of markers.

[c] The fold increase reflects increase from 0.5 cM observed with the control (topmost) cross.

formed a cross between strains SP473 (*mat1-P-smt-s mat2:3-M*) and SP465 (*mat1-M mat2:3-M*). The *smt-s* mutation is a small deletion located distal to *mat1* that reduces the amount of the DSB at *mat1* and consequently reduces switching efficiency (EGEL and GUTZ 1981; BEACH 1983; KLAR, BONADUCE and CAFFERKEY 1991). The reduced level of *mat1* switching due to the *smt-s* mutation in strain SP473 allowed us to keep the *mat1-P* allele unswitched in a significant proportion of cells in the *mat2:3-M* background. Once the *mat1* allele is switched, the cells become stable *M* type. The *smt-s* mutation should not affect our results, since the map expansion in the *mat1* and *his2* interval is also observed in *smt-s* crosses (KLAR and MIGLIO 1986). The *mat2:3-M* allele is a single donor *M*-containing cassette comprised of a fusion of *mat2* and *mat3* resulting in a precise deletion of the *P*-donor locus and the K region (BEACH and KLAR 1984). The results (TABLE 2) show a 12-fold increase in map expansion (5.9 cM) in the *mat1-his2* interval as compared to the value of 0.5 cM observed with control strains (PB30 × SP1001, Table 2). Thus, deletion of the K region and one of the cassettes seems to allow partial map expansion.

To determine whether deletion of the remaining donor cassette results in a further increase in recombination, we constructed and analyzed strains homozygous for a *mat2,3::LEU2* mutation in which the entire *mat2-mat3* region is replaced by the *LEU2* gene. In a cross between SP804 (*mat1-P mat2, 3Δ::leu2*) and SP453 (*mat1-M-smt-s mat2, 3Δ::LEU2*), recombination between *mat1* and *his2* was increased 22-fold compared to the control strain (Table 2). Furthermore, the increased recombination occurred in both the *mat1-mat2* (4.0 cM, 136PD:0NPD:12TT) and the *mat3-his2* (7.2 cM, 130PD:0NPD:22TT) intervals. In a cross between a strain carrying the *mat2,3Δ::LEU2* deletion (SP452) and one carrying the *mat2:3-M* mutation (SP6), recombination was increased 16-fold compared to the wild-type control (Table 2). Again, recombination was increased both in the *mat1-mat2* (4.9 cM, 211PD:0NPD:23TT) and *mat3-his2* (3.9 cM, 212PD:0NPD:18TT) intervals. The observed increase in recombination in the *mat2,3Δ::LEU2* homozygote compared to the *mat2:3-M* homozygote cannot be accounted for by the change in the physical size of the *mat1-his2* interval as the *mat2,3Δ::LEU2* containing interval is actually 0.3 kb shorter than that of the *mat2:3-M* strain (KLAR and MIGLIO 1986). Thus, these results suggest that the remaining donor cassette in *mat2:3-M* strain retains some cold spot activity. However, we cannot rule out the possibility that the increased recombination observed in *mat2,3Δ::LEU2* strains is due to recombination-stimulating activity conferred by *LEU2* (CAO, ALANI and KLECKNER 1990) rather than to deletion of a cold spot.

Interchromosomal (*trans*-) switching occurs inefficiently: If intrachromosomal folding is required for switching, then it is possible that interchromosomal transfer of information may be adversely affected. That is, the donor loci present in one chromosome may not be efficiently used for switching by the *mat1* locus present in the donor-deleted homolog. We tested the possibility of *trans*-switching by analyzing diploid cells of strain SP905 (*mat1Δ-1 mat2 mat3/ mat1-P or M mat2, 3Δ::leu2⁻*). The *mat1Δ-1* is a 7.7-kb deletion encompassing *mat1* gene (see MATERIALS AND METHODS). This strain contains *mat2* and *mat3* loci in *mat1Δ*-containing chromosome. The homolog contains the wild-type *mat1* allele but is deleted for the donor loci. As a control for *cis*-switching in diploids, the cells of strain SP1054 (*h⁹⁰/mat1Δ-1 mat2, 3Δ::leu2⁻*) were similarly analyzed.

A rough estimate of efficiency of switching was obtained by the iodine vapor staining procedure. The switching-proficient, and therefore sporulation-profi-

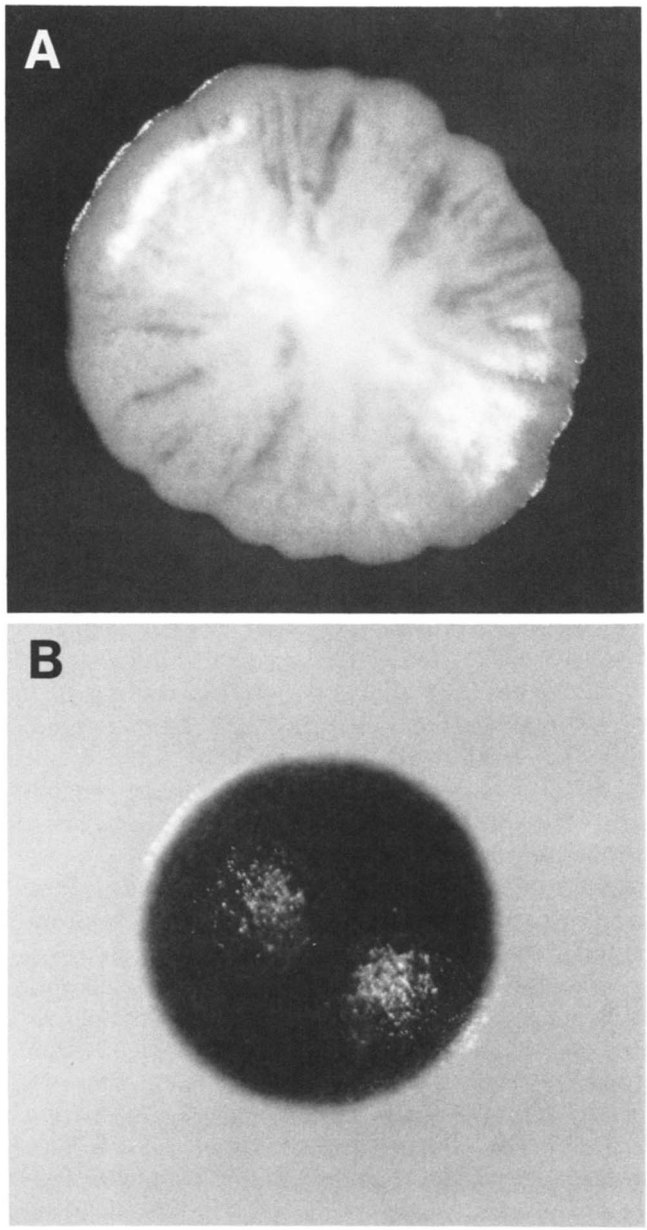

FIGURE 2.—Photographs of yeast colonies stained by exposure to iodine vapors. **A**, Colony grown from a cell of strain SP905. Internal streaks of staining within the colony are due to mating-type switching in *trans*. **B**, Colony grown from a cell of strain SP1054, which is capable of switching in *cis*. Dark color indicates efficient switching. Shiny areas of both colonies are reflections of light used to take pictures.

cient, colonies stain black after exposure to iodine vapors because they accumulate glycogen-like compound during sporulation, while inefficiently switching colonies stain sparingly (BRESCH, MULLER and EGEL 1968). A much reduced efficiency of *trans*-switching is indicated by the reduced iodine vapor staining of SP905 colonies, as compared to those of the control strain SP1054 capable of *cis*-switching (Figure 2).

To better quantitate the efficiency of *trans*-switching, single-cell pedigree analysis was performed as described in MATERIALS AND METHODS. The SP905 strain switched inefficiently as only 4 in 89 cases (4.5%) one-in-four granddaughters of a cell switched. This value of *trans*-switching is 1/18th to that of *cis*-switching observed with the control strain SP1054 which switched in 162 among 198 (81.0%) pedigrees tested.

***swi2* and *swi6* mutants are proficient in utilizing the DSB at *mat1* for meiotic recombination:** It is known that the DSB acts as an efficient initiator of meiotic recombination in donor-deleted *mat2,3Δ::LEU2* strains since such crosses produce 20% tetrads with 3:1 (3*P*:1*M* and 1*P*:3*M*) conversions at *mat1* (KLAR and MIGLIO 1986). Both *swi2*$^-$ and *swi6*$^-$ strains are apparently defective in utilizing the DSB for mitotic *mat1* switching, an event in which donor loci are used as the template for gene conversion (EGEL, BEACH and KLAR 1984). We tested whether *swi2* and *swi6* mutants were defective in utilization of the DSB *per se*. That is, we determined whether they were defective in meiotic conversion at *mat1* as well.

A cross between *swi2*$^-$ strains SP975 (*mat1-P mat2, 3Δ::LEU2*) and SP956 (*mat1-M mat2,3Δ::LEU2*) showed that *mat1* gene conversion occurs efficiently (Table 3). Furthermore, we note that in the *swi2*$^-$ cross the *mat1-his2* interval is 16.4 cM. A cross between *swi6*$^-$ strains SP960 (*mat1-M mat2, 3Δ::LEU2*) and SP962 (*mat1-P mat2, 3Δ::LEU2*) also exhibited an efficient rate of conversion at *mat1* along with an expanded map distance between *mat1* and *his2* (Table 3). Based on these results, we assume that both *swi2* and *swi6* mutant strains are proficient in utilization of the DSB at *mat1* for meiotic conversion. Also, the *mat1-his2* interval in these crosses is similar to that found earlier in *swi*$^+$ *mat2,3Δ::LEU2* strains. Therefore, the cold spot is absent in donor-deleted strains regardless of the *swi* genotype. Secondly, the *swi2* and *swi6* mutants are proficient in utilizing the DSB at *mat1* for meiotic recombination.

Recombination in the *mat2*, K and *mat3* interval is found in *swi6* but not in *swi2* mutants: One possible role of *swi2* and *swi6* in switching could be to render the *mat2* and *mat3* donor loci readily accessible to *mat1* for switching, for example, for precise folding of *mat2* and/or *mat3* onto *mat1*. It is therefore probable that in *swi2* and *swi6* mutants the donor loci in diploids may be allowed to interact interchromosomally, permitting recombination between them and in the K region.

A test of this idea is provided by analyzing sporulation-deficient (spo$^-$) strains that possess defective *mat2* and *mat3* loci. The *mat2-P-B102* and *mat3-M-B406* mutations provide respective mating functions after transfer into *mat1*; however, the resulting zygotic cells are defective for sporulation, as the mutations in both cassettes lie in the sporulation-specific functions

TABLE 3

Effect of *swi* mutations on *mat1* meiotic gene conversion and *mat1-his2* interval in donor-deleted strains

Cross	Genotype	*swi*	Asci classes 2P:2M	3P:1M	1P:3M	Percent conversion	Map distance (cM)[a] *mat1-his2*
SP956 × SP975	*mat1-M mat2,3Δ::LEU2* / *mat1-P mat2,3Δ::LEU2*	$2^-/2^-$	73	12	8	21.5	16.4 (49:0:24)
SP960 × SP962	*mat1-M mat2,3Δ::LEU2* / *mat1-P mat2,3Δ::LEU2*	$6^-/6^-$	69	10	2	15.0	19.5 (30:1:10)

All designations are the same as defined in the legend to Table 2.
[a] Tetrads with *mat1* conversion could not be used for calculating map distances were therefore excluded.

(BRESCH, MULLER and EGEL 1968; KELLY *et al.* 1988). Consequently, such strains switch and mate well but fail to sporulate. The lack of sporulation can be assayed as they are unable to stain when exposed to iodine vapors (BRESCH, MULLER and EGEL 1968). The same set of markers has been used in previous investigations to define and study the cold spot of recombination (EGEL and GUTZ 1981; EGEL 1984; EGEL, WILLER and NIELSEN 1989). The strains were crossed and sporulated, and putative recombinant spores identified both by random spore and tetrad analyses.

A cross between strains SP914 (*h^{90}mat3-M-B406 swi2$^-$*) and SP909 (*h^{90}mat2-P-B102 swi2$^-$*) was subjected to tetrad analysis. Only zygotes resulting from matings of *mat1-P*-containing cells of strain SP914 with *mat1-M*-containing cells of strain SP909 would produce asci. Among 260 tetrads analyzed, 2.7% of the segregants initially stained with iodine vapors (Table 4). However, all such segregants were unstable and produced spo$^-$ progeny on subcloning. Recombinants involving *mat2* and *mat3* loci should have generated the *h^{90} (mat2-P mat3-M) swi2$^-$* genotype and should have established stable spo$^+$ clones owing to the low rate of switching of *swi2$^-$* strains (EGEL, BEACH and KLAR 1984). The segregants that sporulated initially are explained as being generated by crossover events between *mat1* and *mat2*, as well as by meiotic gene conversions of *mat1*. Such events should produce segregants of the genotypes *mat1-P mat2-P-B102 mat3-M* and *mat1-M mat2-P mat3-M-B406*. The wild-type *mat1* allele should be maintained transiently in a significant fraction of such cells, as these strains, being *swi2$^-$*, are defective in switching, thus allowing sporulation initially. However, growth of these cells should produce progeny in which the wild-type *mat1* allele is lost by switching. Such cells should establish spo$^-$ clones on subcloning. Thus, among 260 tetrads analyzed, crossovers or gene conversions between *mat2* and *mat3* were not observed in *swi2$^-$* strains.

In a *swi6$^-$* cross, however, *mat2, mat3* recombinants were obtained. Random spore analysis of a cross between SP948 (*h^{90} mat3-M-B406 swi6$^-$*) and SP965 (*h^{90} mat2-P-B102 swi6$^-$*) produced 6.8% (58 among 850) segregants that were spo$^+$, since they initially stained with iodine vapors. One-third of those tested (12 among 36) established stable spo$^+$ clones. Thus, about 2.3% of random spores were *h^{90}* (*mat2-P mat3-M*) recombinants. The spo$^+$ segregants could result from gene conversion of *mat2* and *mat3* loci and/or from crossing over between these loci. These possibilities were differentiated by tetrad analysis as follows.

A cross between strains SP908 (*h^{90} mat3-M-B406 swi6$^-$*) and SP916 (*h^{90} mat2-P-B102 swi6$^-$*) was subjected to tetrad analysis. Among 248 asci dissected (with 78.0% spore viability), 59 segregants sporulated initially and 13 among them were found to be stable spo$^+$ *h^{90}* segregants (Table 4). The remaining segregants of the asci that contained stable *h^{90}* segregants were further analyzed as described in Materials and Methods to deduce whether the *h^{90}* segregants arose as a result of crossovers between *mat2* and *mat3* and/or by gene conversion of donor loci. By this analysis, three *h^{90}* segregants were assumed to result from crossing over in the K region, since each of the respective tetrads contained another segregant with the reciprocal recombination *mat2-P-B102 mat3-M-B406* product. Two *h^{90}* segregants resulted from gene conversion of *mat2* and six of *mat3*. The origin of the remaining two *h^{90}* segregants could not be determined, as those asci had only two viable spores.

In summary, we found recombination in *mat2-mat3* interval in *swi6$^-$* but not in *swi2$^-$* strains.

The *mat1-his2* interval is expanded in *swi6* mutants: Another difference between *swi2* and *swi6* strains became apparent when the genetic distance between *leu1* and *his2* (Figure 1) was compared in crosses presented in Table 4. That interval is 18.0 cM in the *swi2$^-$* cross, a value comparable to that of *swi2$^+$* wild-type strains (LEUPOLD 1958), but it increased to 52.0 cM in the *swi6$^-$* cross. It was thought unlikely that such a large increase in map distance between *leu1* and *his2* in *swi6$^-$* strains was due to meiotically induced recombination in and around the donor loci, given that only 13 *h^{90}* recombinants were found among 774 segregants analyzed. One source of increased map distance in the *swi6$^-$* genotype could be

TABLE 4

Recombination in the cold spot

Cross	Genotype	*swi*	Asci analyzed	Initial spo$^+$ segregants	Stable spo$^+$ segregants	*leu1-his2* (cM)
SP914 × SP909	*mat3-M-B406* *mat2-P-B102*	$2^-/2^-$	260	2.7%	0%	18.0 (133:3:48)[a]
SP908 × SP916	*mat3-M-B406* *mat2-P-B102*	$6^-/6^-$	248	7.6%	1.7%	52.0 (80:17:37)

[a] Numbers indicate PD:NPD:TT tetrads for *leu1* and *his2* markers.

increased meiotic *mat1* conversion, by interchromosomal interactions of this locus located in the homologs, associated with crossing over of flanking markers. Increased frequency of crossing over was reported earlier in strains containing wild-type *mat1* allele in both homologs (MEADE and GUTZ 1978). To determine the exact amount of recombination in the *mat1-his2* interval, we employed crosses of strains in which at least one of the partners was defective in *mat1* gene conversion.

As a control, a diploid *swi*$^+$ strain SP1002 (*h*^{90}/*mat1-M-smt-o*) was subjected to tetrad analysis. As shown in Table 5, the *mat1-his2* interval was only 0.5 cM in length. A similar distance of 0.9 cM was observed in the *swi2*$^-$ SP1003 (*h*^{90}/*mat1-M-smt-o*) strain. The *swi6*$^-$ strain SP1004 (*h*^{90}/*mat1-M-smt-o*), however, exhibited an increased map distance of 5.5 cM. Therefore, this interval increased about 11-fold in the *swi6*$^-$ strain when compared to the value obtained with the *swi*$^+$ (SP1002) strain.

It appears that in *S. pombe*, analyses of "zygotic asci" (resulting from a recently mated zygotic cells) generally produce a map distance twice of that observed with "azygotic asci" (EGEL, WILLER and NIELSEN 1989). All three crosses presented in this section, however, involved analysis of "azygotic asci" that were produced by cells that were already diploid (hence capable of sporulation directly without further mating). Therefore, we assayed the *leu1-mat1* and the *mat1-his2* intervals in *swi6*$^-$ strains by analyzing zygotic asci resulting from a cross between strains SP107 (*h*^{90} *swi6*$^-$) and SP997 (*mat1-PΔ17 swi6*$^-$). As shown in Table 5, as compared to the crosses presented above involving azygotic asci, the *leu1-mat1* interval increased to 14.7 cM in the *swi6*$^-$ cross. But most significantly, the *mat1-his2* interval also increased to 14.6 cM, a value similar to that obtained in crosses with donor-deleted strains (Table 3; KLAR and MIGLIO 1984). It is a formal possibility that the DSB in the *h*^{90} chromosome in strain SP107 may cause map expansion in that cross. This possibility was ruled out by the result obtained with a cross between strains SP931 (*mat1-M-smt-o swi6*$^-$) and SP998 (*mat1PΔ17::LEU2 swi6*$^-$) also exhibiting map expansion (Table 5). As another *swi*$^+$ control involving analysis of zygotic asci (partial data presented in Table 2), a cross between strains PB30 (*mat1-PΔ17*) and SP1001 (*mat1-M-smt-o*) is presented in Table 5 for comparison.

In summary, the *mat1-his2* interval was not significantly affected in *swi2*$^-$ strains, but it increased as much as 29-fold in *swi6*$^-$ strains. The *leu1-mat1* interval was unaffected by the *swi* genotype.

DISCUSSION

The *mat1* locus acts as a hot spot of recombination both in mitosis and in meiosis. Mating-type switching is one such result of efficient recombination in mitosis. The other is efficient homozygosis of all markers located distal to *mat1* in diploids; the homozygosis occurs in about 2% of cells during each division (EGEL 1981). In meiosis, the *mat2* and *mat3* donor- deleted strains undergo efficient meiotic gene conversion at *mat1*, with 20% of asci producing aberrant gene conversion events at *mat1* (KLAR and MIGLIO 1986). In addition, meiotic map distance of flanking markers increases significantly when both homologs contain the switching-proficient *mat1* allele (LEUPOLD 1958; MEADE and GUTZ 1978; this paper). All of these recombination events are now thought to be promoted by the DSB at *mat1*. In contrast, in the adjoining *mat2*, *mat3* and the intervening 15 kb K region, a total lack of meiotic recombination is observed (EGEL 1984). It is possible that this meiotic cold spot has nothing to do with mating-type switching which occurs in mitotically dividing cells. However, location of the cold spot between the donor loci essential for switching and the lack of any essential gene in this region (BEACH and KLAR 1984; KLAR and MIGLIO 1984) prompted us to investigate the cold spot in certain *swi*$^-$ mutants. This study addresses the reason for this cold spot of recombination in relation to the process of mating-type interconversion.

The observation that deletion of the *mat2*, *mat3* and K region allows a six- to ninefold increased meiotic recombination in the *mat1-his2* interval suggests that the cold spot inhibits recombination even outside of the *mat2* and *mat3* interval (KLAR and MIGLIO 1986). The present study has extended this result by showing that when one of the donor loci plus the K region are deleted, there is partial removal of the cold spot of

TABLE 5

Effect of *swi* mutations on meiotic recombination in the *rat* region

Strain	Genotype	*swi*	Asci type	Map distance (cM)		
				leu1-his2	*leu1-mat1*	*mat1-his2*
SP1002	h^{90}/*mat1-M-smt-o*	+/+	Azygotic	7.3 (98:1:10)	6.5 (98:1:8)	0.5 (107:0:1)
SP1003	h^{90}/*mat1-M-smt-o*	$2^-/2^-$	Azygotic	9.5 (80:0:21)	8.6 (91:0:19)	0.9 (108:0:2)
SP1004	h^{90}/*mat1-M-smt-o*	$6^-/6^-$	Azygotic	12.0 (78:0:24)	7.0 (86:0:15)	5.5 (90:0:11)
SP107 × SP997	h^{90} *x* *mat1-PΔ17*	$6^-/6^-$	Zygotic	28.0 (76:4:48)	14.7 (94:2:23)	14.6 (95:2:23)
SP931 × SP998	*mat1-M-smt-o x* *mat1-PΔ17*	$6^-/6^-$	Zygotic	ND	ND	9.1 (72:0:16)
PB30 × SP1001	*mat1P-Δ17 x* *mat1-M-smt-o*	+/+	Zygotic	19.0 (80:2:31)	18.5 (80:2:29)	0.5 (439:0:4)

h^{90} is a wild-type *mat* region, while *mat1-M-smt-o* and *mat1-PΔ17* are defective in switching because they possess deletions of essential *cis*-acting sites. All designations are defined in Table 2. ND = not determined.

recombination. A further increase occurs when the remaining *mat* cassette is deleted. As compared to the value of 0.5 cM observed in a control cross, the donor-deleted strains exhibited a 22-fold increased recombination in the *mat1-his2* interval. The increased recombination in donor-deleted strains was found to be equally distributed in intervals to the left of *mat2* and to the right of *mat3*.

The second finding is that *mat1* switching occurs primarily intrachromosomally. The donor loci located in one chromosome efficiently donate information for switching to the *mat1* locus located in the same chromosome and very inefficiently to the locus residing in the homolog. This result suggests that switching does not occur through a diffusible intermediate; rather, there appears to be a physical interaction between *mat1* and the donor loci.

Another important result offers an explanation for the rather paradoxical properties of the *swi2* and *swi6* switching-defective mutants. That is, these mutants contain a normal amount of the DSB, yet they switch inefficiently. We had originally suggested that these mutants are defective in the initial utilization of the DSB for mating-type switching (EGEL, BEACH and KLAR 1984). In this study, we show that utilization of the DSB for meiotic conversion at *mat1* is not affected in those mutants, as efficient gene conversions at *mat1* are found in donor-deleted strains. We imagine that utilization of the DSB for *mat1* switching in mitosis is adversely affected in *swi6* mutants because of defective accessibility of donor loci for switching. The main finding supporting this idea is that much increased recombination within and around the cold spot is found in a *swi6* mutant. Both gene conversions of *mat2* and *mat3* as well as crossover events in the K region were found. The strains containing a *swi2–73* mutation, however, behaved identically to wild-type strains in terms of the lack of recombination in the cold spot.

Based on these results, we propose a model of intrachromosomal folding (looping) that promotes *mat1* switching and prohibits meiotic recombination in the cold spot. The looping model is the simplest interpretation of our results. It may be promoted by a higher order chromatin structure causing a specific organization of the mating-type region. We propose that this looping may be required to make the donor loci readily accessible to *mat1* for switching and that *swi6* plays an essential role in folding. We predict that mating cell type of the cell influences looping such that the specific donor locus is preferentially made available for recombination. We note that a remarkably short sequence homologies of 59 and 135 bp (flanking the allele-specific 1.1-kb sequence) present in all cassettes (KELLY *et al.* 1988) are efficiently used for pairing for gene conversion during switching. The postulated folding/looping perhaps facilitates interactions between these short stretches of sequences.

In this context, we note that an aberrant *mat3:1* fusion cassette is found in a small proportion of cells of *swi6* mutant strains (EGEL, BEACH and KLAR 1984). Such a fusion cassette is normally found in h^{+N} and h^{-U} rearrangements at *mat1* that result from a large 17-kb gene conversion event by which *mat2*, K and *mat3* sequences are transmitted to *mat1* (BEACH and KLAR 1984). They are thought to be the result of resolution errors in H2 sequence; that is, after recombination starts by copying *mat3*, it is completed at a significant frequency by resolving in the H2 region of *mat2* instead of that at *mat3*. We imagine that the folding defect in *swi6* mutants may affect both the initial interaction of *mat1* with the donor loci as well as the step of resolution as the structure is not properly held together during recombination. Such a defect

could produce the aberrant *mat3:1* fusion cassette.

A mutation called *rik1* (for recombination in the K region), has also been shown recently to allow recombination in the K region without affecting recombination in the *mat1-mat2* and *mat3-his2* intervals (EGEL, WILLER and NIELSEN 1989). The *rik1* mutation maps to chromosome *III*, 9.0 cM from *ade6*. *swi6* clearly is a different gene, as we found it to be unlinked to *ade6* (8PD:8NPD:20TT). The *rik1-1* mutant strain stains evenly gray with iodine vapors; thus, it must switch much more efficiently than a *swi6* mutant, which stains only lightly. In addition, the spore viability of *rik1-1* mutants is much reduced (49.7% at 25°, EGEL, WILLER and NIELSEN 1989) as compared to that of the *swi6*$^-$ strain (78.0%, this paper). The *rik1* mutation has pleiotropic defects and it is speculated that the *rik1* protein may be required for heterochromatization throughout the silent domain of the mating-type region. We propose that the *swi6* function is required more directly for mating-type switching, perhaps by regulating accessibility of donor loci for *mat1* recombination.

There are other known regions of crossover suppression in diverse organisms. Complementing auxotrophs for thiamine-, nicotinamide- and acetate-requirement are nearly completely linked to mating type and to each other in *Chlamydomonas reinhardii* (GILLHAM 1969). Two additional cases of recombination suppression in sequences of *S. cerevisiae* have been described recently. The 200 genes of tandemly repeated ribosomal DNA clusters recombine sparingly during mitotic growth despite the sequence reiteration. In topoisomerase I and II mutants, a 50–200-fold higher frequency of mitotic recombination in ribosomal DNA relative to *TOP*$^+$ controls is observed (CHRISTMAN, DIETRICH and FINK 1988). Similarly, the repetitive delta sequences present in about 100 copies distributed throughout the genome are downregulated for recombination. A mutation in a gene encoding a protein homologous to bacterial type I topoisomerase allows recombination in delta sequences (WALLIS *et al.* 1989). Centromere regions (MATHER 1939; NAKASEKO *et al.* 1986) and a universal reduction of exchanges in heterochromatin (JONES 1987) are other noteworthy cases of cold spots of recombination. Seemingly, many different mechanisms can regulate recombination. While this work was in progress, we learned from an independent study that *swi6* mutation affects recombination in the mating-type region (A. LORENTZ, L. HEIM and H. SCHMIDT, personal communication).

We are thankful to B. ARCANGIOLI, S. BROWN, D. GARFINKEL, J. SINGH, G. THON, and J. STRATHERN for interesting discussions, R. FISHEL, D. GARFINKEL and A. ARTHUR for reading the paper, R. EGEL for an unanonymous critical review, R. FREDERICKSON for the artwork, and P. HALL for preparing the manuscript. Research is sponsored by the National Cancer Institute, Department of Health and Human Services, under contract N01-CO-74101 with ABL. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

LITERATURE CITED

ARCANGIOLI, B., and A. J. S. KLAR, 1991 A novel switch-activating site (SAS1) and its cognate binding factor (SAP1) required for efficient *MAT1* switching in *S. pombe*. EMBO J. (in press).

BEACH, D. H., 1983 Cell type switching by DNA transposition in fission yeast. Nature **305:** 682–688.

BEACH, D. H., and A. J. S. KLAR, 1984 Rearrangements of the transposable mating-type cassettes of fission yeast. EMBO J. **3:** 603–610.

BEACH, D., and P. NURSE, 1981 High-frequency transformation of the fission yeast *Schizosaccharomyces pombe*. Nature **290:** 140–142.

BRESCH, C., G. MULLER and R. EGEL, 1968 Genes involved in meiosis and sporulation of a yeast. Mol. Gen. Genet. **102:** 301–306.

CAO, L., E. ALANI and N. KLECKNER, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:** 1089–1101.

CHRISTMAN, M. F., F. S. DIETRICH and G. R. FINK, 1988 Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. Cell **55:** 413–425.

EGEL, R., 1977 Frequency of mating-type switching in homothallic fission yeast. Nature **266:** 172–174.

EGEL, R., 1981 Mating-type switching and mitotic crossing-over at the mating-type locus in fission yeast. Cold Spring Harbor Symp. Quant. Biol. **45:** 1003–1007.

EGEL, R., 1984 Two tightly linked silent cassettes in the mating-type of *Schizosaccharomyces pombe*. Curr. Genet. **8:** 199–203.

EGEL, R., 1989 Mating-type genes, meiosis and sporulation, pp. 31–73 in *Molecular Biology of the Fission Yeast*, edited by A. NASIM, P. YOUNG and B. JOHNSON. Academic Press, New York.

EGEL, R., D. H. BEACH and A. J. S. KLAR, 1984 Genes required for initiation and resolution steps of mating-type switching in fission yeast. Proc. Natl. Acad. Sci. USA **81:** 3481–3485.

EGEL, R., and B. EIE, 1987 Cell lineage asymmetry in *Schizosaccharomyces pombe*: unilateral transmission of a high-frequency state of mating-type switching in diploid pedigrees. Curr. Genet. **12:** 429–433.

EGEL, R., and H. GUTZ,, 1981 Gene activation by copy transposition in mating-type switching of a homothallic fission yeast. Curr. Genet. **3:** 5–12.

EGEL, R., M. WILLER and O. NIELSEN, 1989 Unblocking of meiotic crossing-over between the silent mating-type cassettes of fission yeast, conditioned by the recessive, pleiotropic mutant *rik1*. Curr. Genet. **15:** 407–410.

ENGELKE, U., L. GRABOWSKI, H. GUTZ, L. HEIM and H. SCHMIDT, 1987 Molecular characterization of h^- mutants of *Schizosaccharomyces pombe*. Curr. Genet. **12:** 535–542.

GILLHAM, N., 1969 Uniparental inheritance in *Chlamydomonas reinhardii*. Am. Nat. **103:** 355–388.

GUTZ, H., H. HESLOT, U. LEUPOLD and N. LOPRIENO, 1974 *Schizosaccharomyces pombe*, pp. 395–446 in *Handbook of Genetics*, edited by R. C. KING. Plenum Press, New York.

JONES, G. H., 1987 Chiasmata, pp. 213–244 in *Meiosis*, edited by P. B. MOENS. Academic Press, London.

KELLY, M., J. BURKE, M. SMITH, A. KLAR and D. BEACH, 1988 Four mating-type genes control sexual differentiation in the fission yeast. EMBO J. **7:** 1537–1547.

KLAR, A. J. S., 1987 Differentiated parental DNA strands confer

developmental asymmetry on daughter cells in fission yeast. Nature **326:** 466–470.

KLAR, A. J. S., 1989 The interconversion of yeast mating type: *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, pp. 671–691 in *Mobile DNA*, edited by D. E. BERG and M. W. HOWE. American Society for Microbiology, Washington, D.C.

KLAR, A. J. S., 1990 The developmental fate of fission yeast cells is determined by the pattern of inheritance of parental and grandparental DNA strands. EMBO J. **9:** 1407–1415.

KLAR, A. J. S., M. J. BONADUCE and R. CAFFERKEY, 1991 The mechanism of fission yeast mating type interconversion: seal/replicate/cleave model of replication across the double-stranded break site at *mat1*. Genetics **127:** 489–496.

KLAR, A. J. S., and L. M. MIGLIO, 1986 Initiation of meiotic recombination by double-strand DNA breaks in *S. pombe*. Cell **46:** 725–731.

LEUPOLD, U., 1950 Die vererbung von homothallie und heterothallie bei *Schizosaccharomyces pombe*. C. R. Trav. Lab. Carlsberg Ser. Physiol. **24:** 381–480.

LEUPOLD, U., 1958 Studies on recombination in *Schizosaccharomyces pombe*. Cold Spring Harbor Symp. Quant. Biol. **23:** 161–170.

MATHER, K., 1939 Crossing-over and heterochromatin in the X chromosome of *Drosophila melanogaster*. J. Genet. **33:** 207–235.

MEADE, J. H., and H. GUTZ, 1978 Influence of the *mat1-M* allele on meiotic recombination in the mating-type region of *Schizosaccharomyces pombe*. Genetics **88:** 235–238.

MIYATA, H., and M. MIYATA, 1981 Mode of conjugation in homothallic cells of *Schizosaccharomyces pombe*. J. Gen. Appl. Microbiol. **27:** 365–371.

MORENO, S., A. KLAR and P. NURSE, 1991 Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol. **194:** 795–823.

NAKASEKO, Y., Y. ADACHI, S. FUNAHASHI, O. NIWA and M. YANAGIDA, 1986 Chromosome walking shows a highly homologous repetitive sequence present in all the centromere regions of fission yeast. EMBO J. **5:** 1011–1021.

NIELSEN, O., and R. EGEL, 1989 Mapping the double-strand breaks at the mating-type locus in fission yeast by genomic sequencing. EMBO J. **8:** 269–276.

PERKINS, D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:** 607–626.

SCHMIDT, H., P. KAPITZA and H. GUTZ, 1987 Switching genes in *Schizosaccharomyces pombe*: their influence on cell viability and recombination. Curr. Genet. **11:** 303–308.

SZOSTACK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. STAHL, 1983 The double-standard-break repair model for recombination. Cell **33:** 25–35.

VAN SOLINGEN, P., and J. B. VAN DER PLAAT, 1977 Fusion of yeast protoplasts. J. Bacteriol. **130:** 946–947.

WALLIS, J. W., G. CHREBET, G. BRODSKY, M. ROLFE and R. ROTHSTEIN, 1989 A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. Cell **58:** 409–419.

Communicating editor: G. S. ROEDER