

Genetic and Physical Analysis of the *M26* Recombination Hotspot of *Schizosaccharomyces pombe*

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Manuscript received October 7, 1987

Revised copy accepted March 3, 1988

ABSTRACT

The *ade6-M26* mutation of *Schizosaccharomyces pombe* has previously been reported to stimulate *ade6* intragenic meiotic recombination. We report here that the *ade6-M26* mutation is a single G → T nucleotide change, that *M26* stimulated recombination within *ade6* but not at other distant loci, and that *M26* stimulated meiotic but not mitotic recombination. In addition, *M26* stimulated recombination within *ade6* when *M26* is homozygous; this result demonstrates that a base-pair mismatch at the *M26* site was not required for the stimulation. These results are consistent with the *ade6-M26* mutation creating a meiotic recombination initiation site.

HOMOLOGOUS recombination involves the exchange of genetic information anywhere along homologous DNA molecules. In numerous organisms certain regions or sites exhibit an elevated frequency of recombination (WHITEHOUSE 1982). These special sites are presumed to stimulate some rate-limiting step in recombination and, therefore, provide a useful focus for recombination studies.

In the fission yeast *Schizosaccharomyces pombe*, the *ade6-M26* mutation has been proposed to create a site that stimulates meiotic recombination within *ade6*. Compared to the closely linked *ade6-M375* mutation, *ade6-M26* yields up to 15 times more prototrophic recombinants in intragenic crosses (GUTZ 1963, 1971). When crossed with wild type or with other *ade6* alleles, *ade6-M26* yields a relatively high frequency of gene conversion tetrads (3–5%), about ten times the frequency observed with other *ade6* alleles. In addition, these convertant tetrads show extreme disparity; the mutation at *ade6-M26* is predominantly converted to wild type, with 3+ : 1 *M26* tetrads being about 12 times more frequent than 1+ : 3 *M26* tetrads. This is in contrast to the parity of gene conversion observed at other *ade6* mutant sites (GUTZ 1971). In crosses containing multiple *ade6* mutations, *M26* “pulls” adjacent sites into its conversion pattern and produces double- and triple-site co-conversions (GUTZ 1971). This effect is observed with *ade6* alleles on both sides of *M26*.

We have begun a genetic and physical analysis of *M26* to further our understanding of this marker effect. We have tested whether a base-pair mismatch at the *M26* site is required for recombination stimulation. The effect of *M26* on mitotic recombination

and on recombination at loci distant from *ade6* was also investigated. We also report the cloning of *ade6-M26* and the nature of the *M26* mutation.

MATERIALS AND METHODS

Media: Yeast-extract agar (YEA), yeast extract liquid (YEL), minimal agar (MMA), malt-extract agar (MEA), and synthetic sporulation agar (SPA) were prepared as described by GUTZ *et al.* (1974). Modified EMM2 (minimal liquid) was prepared as described by NURSE (1975).

Meiotic crosses: Meiotic recombinant frequencies were determined by random spore analysis. Standard crosses were performed by growing each parent in YEL at 30° to approximately 5×10^7 cells/ml. One milliliter of each parental culture was mixed. The cells were pelleted, washed once with 5 ml saline (0.85% NaCl), and resuspended in 0.5 ml of saline. For the crosses listed in Table 2, each parent was grown on YEA for 2 days at 32°. A half-loopful of cells ($1-3 \times 10^7$ cells) of each strain to be mated was suspended in 0.5 ml saline. Then 0.2 ml of the cross mixture was transferred to the surface of a 5 ml MEA slant containing appropriate supplements at 50 µg/ml. The slants were incubated at 25° for 3–5 days. Sporulated zygotes and unmated cells were harvested from the MEA slant in 2 ml sterile distilled water. Glusulase (Dupont) (5 or 10 µl) was added, and the suspension incubated 6–16 hr at 30° to liberate free spores from the asci. Two milliliters of 60% ethanol were added, and the mixture incubated at room temperature for 30 min to kill any remaining vegetative cells. Spores were pelleted, washed with 5 ml sterile distilled water, and resuspended in 1 ml of sterile distilled water. Dilutions of the spore suspensions in sterile water were plated on the appropriate medium.

Strains: *S. pombe* strains used in this work are listed in Table 1. A genetic map of the *ade6* locus showing the approximate location of the mutations used in this study is presented in Figure 1. Stable nonsporulating diploids heteroallelic at *ade6* were constructed by a modification of the procedure described by FLORES DA CUNHA (1970). Approximately 5×10^7 cells each of strains GP42 and GP128 or GP42 and GP130 were mixed in 0.5 ml saline in an Eppendorf tube. The cells were pelleted 10 sec in an

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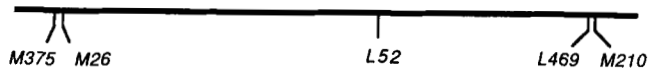


FIGURE 1.—Genetic map of the *ade6* locus showing the approximate locations of the mutations used in this work.

TABLE 1
S. pombe strains

SMITH laboratory strain	Genotype	Source or derivation
GP3	<i>h⁻ ade6-M26 sup9</i>	S. GOLDMAN
GP4	<i>h⁺ ade6-M210</i>	S. GOLDMAN
GP6	<i>h⁺ ade6-M375</i>	S. GOLDMAN
GP8	<i>h⁻ ade6-M26-M210</i>	S. GOLDMAN
GP14	<i>h⁺ ade6-L52</i>	S. GOLDMAN
GP16	<i>h⁺ ade6-M26-L52</i>	S. GOLDMAN
GP17	<i>h⁺ ade6-M26-M210</i>	GP8 × GP19
GP19	<i>h⁺</i>	S. GOLDMAN
GP23	<i>h⁻ ade6-M26</i>	S. GOLDMAN
GP24	<i>h⁺ ade6-M26</i>	GP3 × GP19
GP25	<i>h⁻ sup9</i>	GP3 × GP19
GP26	<i>h⁻ ade6-L52 sup9</i>	GP14 × GP25
GP27	<i>h⁺ ade6-M210 sup9</i>	GP4 × GP25
GP28	<i>h⁻ ade6-M26-L52 sup9</i>	GP16 × GP25
GP29	<i>h⁺ ade6-M26-M210 sup9</i>	GP17 × GP25
GP31	<i>h⁻ ura4-294</i>	V. ZAKIAN
GP42	<i>h⁺ ade6-M210 ura4-294</i>	GP4 × GP31
GP51	<i>h⁹⁰ ade6-M26</i>	GP24
GP52	<i>h⁹⁰ ade6-M375</i>	GP6
GP57	<i>h⁹⁰ ade6-M26 ura4-294</i>	GP31 × GP51
GP58	<i>h⁹⁰ ade6-M375 ura4-294</i>	GP31 × GP52
GP66	<i>h⁹⁰ ade6-M26</i> (pade6-L469)	GP57 + pade6-L469
GP67	<i>h⁹⁰ ade6-M375</i> (pade6-L469)	GP58 + pade6-L469
GP68	<i>h⁺ ade6-M210 ura4-294</i> <i>h⁺ ade6-M26 arg1-2</i>	GP42 × GP128
GP69	<i>h⁻ ade6-M210 ura4-294</i> <i>h⁻ ade6-M375 arg1-2</i>	GP42 × GP130
GP73	<i>h⁻ pro2-1</i>	J. KOHLI
GP75	<i>h⁻ ura2-10</i>	J. KOHLI
GP77	<i>h⁻ leu2-120</i>	J. KOHLI
GP83	<i>h⁻ arg4-55</i>	J. KOHLI
GP85	<i>h⁻ arg5-189</i>	J. KOHLI
GP88	<i>h⁺ ade6-M210 arg3-124</i>	J. KOHLI
GP90	<i>h⁻ ade6-M375 pro2-1</i>	GP6 × GP73
GP92	<i>h⁻ ade6-M26 pro2-1</i>	GP24 × GP73
GP93	<i>h⁺ ade6-M210 leu2-120</i>	GP4 × GP77
GP96	<i>h⁻ ade6-M375 ura2-10</i>	GP6 × GP75
GP98	<i>h⁻ ade6-M26 ura2-10</i>	GP24 × GP75
GP99	<i>h⁺ ade6-M210 arg4-55</i>	GP4 × GP83
GP102	<i>h⁻ arg1-14</i>	A. NASIM
GP103	<i>h⁻ arg1-2</i>	A. NASIM
GP105	<i>h⁻ arg1-230</i>	H. AMSTUTZ
GP126	<i>h⁺ ade6-M210 arg1-230</i>	GP4 × GP105
GP127	<i>h⁻ ade6-M26 arg1-14</i>	GP24 × GP102
GP128	<i>h⁻ ade6-M26 arg1-2</i>	GP24 × GP103
GP129	<i>h⁻ ade6-M375 arg1-14</i>	GP6 × GP102
GP130	<i>h⁻ ade6-M375 arg1-2</i>	GP6 × GP103
GP142	<i>h⁺ ade6-M210 arg1-2</i>	GP4 × GP103
GP143	<i>h⁻ ade6-M375 arg1-230</i>	GP6 × GP105
GP144	<i>h⁻ ade6-M26 arg1-230</i>	GP24 × GP105
GP153	<i>h⁻ ade6-M375 arg5-189</i>	GP6 × GP85
GP155	<i>h⁻ ade6-M26 arg5-189</i>	GP24 × GP85

Eppendorf microcentrifuge, resuspended with 10 μ l saline and spotted on SPA. The plate was incubated at 25° for 16 hr by which time many nonsporulated zygotes were present. The mating mixtures were transferred to 50 ml EMM2 + adenine (100 μ g/ml), shaken at 30° for 48 hr to select for growth of diploids via intergenic complementation of *arg1-2* and *ura4-294*, and plated on MMA + adenine. After 4 days growth at 30°, the plates were treated with iodine vapors to distinguish sporulating diploids from nonsporulating (stable) diploids or recombinant haploids (GUTZ *et al.* 1974). Colonies not staining with iodine were streaked on YEA containing magdala red (phloxin B, 0.001%; Sigma) to distinguish haploids from diploids (GUTZ *et al.* 1974). Diploidy was also verified by microscopy; diploid cells are markedly larger than haploid cells.

Strains GP57 and GP58 were transformed with Ura⁺ with plasmid pade6-L469 to generate strains GP66 and GP67, respectively. Transformation of *S. pombe* was carried out by the spheroplasting procedure described by BEACH, PIPER and NURSE (1982) with slight modifications. Harvested cells were incubated in 1.2 M sorbitol, 20 mM sodium citrate pH 5.6, 40 mM EDTA, 30 mM 2-mercaptoethanol for 10 min at 30°, collected by centrifugation, resuspended at 3 × 10⁸ cells/ml in 1.2 M sorbitol, 50 mM sodium citrate pH 5.6, 5 mg/ml Novo SP234 (Novo Labs, Wilton, Connecticut) and treated further as described by BEACH, PIPER and NURSE (1982).

Homothallic strains GP51 and GP52 were derived by spontaneous reversion of the *h⁺* heterothallic strains GP24 and GP6, respectively (*h⁺* strains revert to homothallic *h⁹⁰* at a frequency of 10⁻⁵; BEACH and KLAR 1984). Strains GP24 and GP6 were streaked on MMA + adenine, the plates incubated at 32° for 5 days, then treated with iodine vapors to detect iodine-staining (sporulating) *h⁹⁰* revertants.

Crosses to test for localized *ade6-M26* action: Standard crosses were performed on MEA slants and spores plated onto appropriately supplemented (50–100 μ g/ml) or non-supplemented MMA plates to determine recombinant frequencies.

Crosses to test the effect of *ade6-M26* homozygosity: Standard crosses were performed on MEA slants. Since diploid spores (occasionally present after zygote sporulation) would have complicated the analysis of crosses containing the complementing *L52* and *M210* alleles (GUTZ 1963), Ade⁺ recombinants were scored by plating spores on MMA + 0.014% *m*-fluorophenylalanine (*m*-FPA, Sigma). *m*-FPA induces mitotic haploidization of diploids (KOHLI *et al.* 1977; ADONDI and HESLOT 1970) and in our experiments resulted in the selective loss of diploid colonies growing on MMA due to intragenic complementation and had no effect on the efficiency of plating of haploid parents or recombinants (data not shown).

Plasmids: The plasmids used in this work are presented in Figure 2. pSPa6 (14 kb) contains a 6.4-kb partial *Sau3A* fragment containing the *S. pombe ade6⁺* region inserted into the *Bam*HI site of pFL20 (from LOUISE CLARK, via JURG KOHLI). pFL20, and the derivatives described here, contain an *S. pombe ars*, a functional *Saccharomyces cerevisiae URA3* gene (which complements *S. pombe ura4* mutations) and is maintained at a copy number of about 80 per haploid cell (HEYER, SIPCZKI and KOHLI 1986; LOSSON and LACROUTE 1983). pade6-L469 (9.2 kb, from P. SZANKASI, W.-D. HEYER, and J. KOHLI) contains a 3-kb *Pvu*II-*Sau*3A fragment containing *ade6-L469* substituted for the *Pvu*II-*Bam*HI fragment of pFL20. The generation of pASP1 is described below.

Cloning and sequence analysis of *ade6-M26*: *S. pombe*

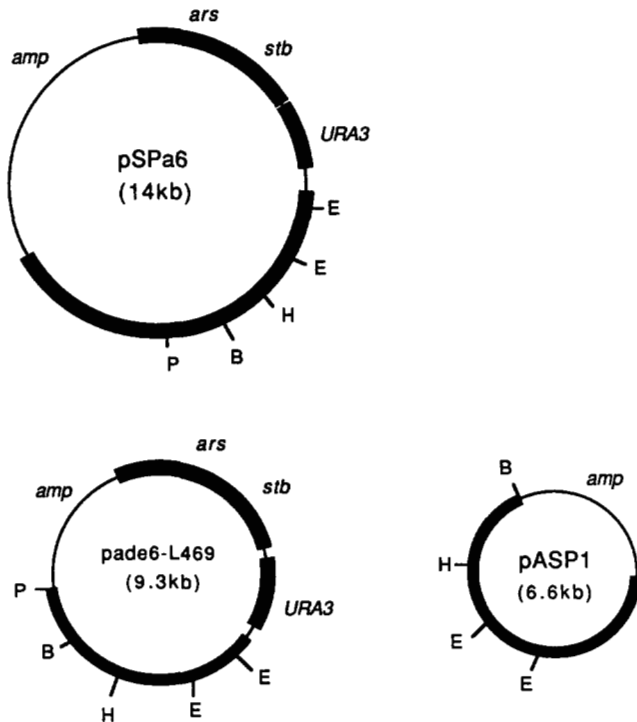


FIGURE 2.—Plasmids used in this study. For details of plasmid derivations see MATERIALS AND METHODS. Thin lines represent pBR322 DNA (in pSPa6 and pade6-L469) or pUC19 DNA (in pASP1) including the ampicillin-resistance determinant *amp*. Thick lines represent cloned inserts. *Ars* and *stb* are elements from *S. pombe* for replication and stability of the plasmids (HEYER, SIPCZKI and KOHLI 1986). *URA3* is the wild-type gene from *S. cerevisiae*. Remaining inserts contain *S. pombe ade6* DNA. Only relevant restriction sites are presented to show the orientations of the inserts: B, *Bam*HI; E, *Eco*RI; H, *Hind*III; P, *Pvu*II. Transcription of *ade6* initiates approximately 90 bp upstream of the *Bam*HI site and proceeds toward the *Hind*III site, *i.e.*, in the counter-clockwise direction as diagrammed here (P. SZANKASI and J. KOHLI, personal communication).

DNA was isolated from the *ade6-M26* strain GP23 as described by BEACH and KLAR (1984). The DNA was digested with *Bam*HI and ligated into the *Bam*HI site of pUC19 (YANISCH-PERRON, VIEIRA and MESSING 1985). The ligation mixture was used to transform *Escherichia coli* strain JM107 (YANISCH-PERRON, VIEIRA and MESSING 1985) to ampicillin-resistance by the method of HANAHAN (1983). Ampicillin-resistant transformant colonies were screened by colony hybridization (HANAHAN and MESELSON 1980), using the 865 bp *Eco*RI fragment from pSPa6 as an *ade6* probe. One positive colony contained a 3.9-kb insert into pUC19; this plasmid was designated pASP1. The supercoiled plasmid DNA was sequenced by the dideoxy method of CHEN and SEEBURG (1985) using an M13 primer (from MAGGIE INSLEY, Zymogenetics, Seattle).

Replacement of the M26 mutation onto the chromosome: Spheroplasts (100 μ l) prepared from strain GP18 (*h⁻ leu1-32*) were co-transformed with 1 μ g each of the 815 bp *Bam*HI-*Hind*III fragment from pade6-M26 and plasmid pDB248' (which carries the *S. cerevisiae LEU2* gene, which complements the *S. pombe leu1-32* mutation; BEACH, PIPER and NURSE 1982). Transformed spheroplasts were gently plated on MMA plates containing 1.2 M sorbitol and 10 μ g/ml adenine. On this medium, *ade6⁺* colonies are white, whereas *ade6* mutant colonies are red. Plates were

incubated at 32° for 6 days. Of the 4500 *Leu⁺* transformants, 2 red *Ade⁻* colonies were obtained. The colonies were purified by streaking three times on YEA, then tested in crosses with strains GP29 and GP4 for the presence of the opal-suppressible *ade6* mutation and for recombination hotspot activity, respectively.

Test of mitotic vs. meiotic action of *ade6-M26*: (1) *Diploids*—strains GP68 and GP69, grown in EMM2 + adenine, were diluted to about 50 cells/100 mls. Twenty-four 1-ml cultures were incubated at 30° for 5 days. Five of the twenty-four GP68 cultures and 7 of the 24 GP69 cultures showed growth, to a final cell density of about 2×10^7 /ml. Five positive cultures of each strain were diluted appropriately and plated on MMA or MMA + adenine to determine the frequency of *Ade⁺* recombinants in each culture. The frequencies listed in Table 5 correspond to the cultures with the median recombinant frequency for each set. For the determination of meiotic recombinant frequencies, standard crosses were performed. (2) *Chromosome \times plasmid*—strains GP66 and GP67, grown in EMM2 + adenine to about 2×10^6 cells/ml, were diluted to about 2 cells/ml. Seven 5-ml cultures of each strain were grown at 30° for 5 days to about 2×10^7 cells/ml, diluted, and plated on MMA + uracil or MMA + uracil + adenine to determine the frequency of *Ade⁺* recombinants in each culture. The frequencies listed in Table 5 correspond to the cultures with the median recombinant frequency for each set. For the determination of meiotic recombinant frequencies, we centrifuged the cultures used for the mitotic analysis, washed the cells with saline and spotted them at high cell density on SPA + adenine. The SPA plates were incubated for 2 days at 25°, by which time the spots contained many asci due to homothallic meiotic selfings. The spottings were harvested from the plates and treated as a standard mating mixture to isolate free spores. Appropriate dilutions were plated on MMA + uracil or MMA + uracil + adenine to determine the frequency of recombinant *Ade⁺* spores.

RESULTS

Localized action of *ade6-M26*: To determine whether the *ade6-M26* allele acts only locally or throughout the genome, we measured recombination at several loci when *M26* or the control allele *M375* was present in the cross. Initially we tested whether *ade6-M26* affects intragenic recombination at the *arg1* locus, approximately 80 cM distal to *ade6* on the same arm of chromosome III (KOHLI *et al.* 1977). The *Arg⁺* and *Ade⁺* recombinant frequencies obtained by crossing appropriate *ade6 arg1* containing strains are listed in Table 2. As expected, the presence of *ade6-M26* increased the *Ade⁺* recombinant frequency an average of 10-fold above that of *ade6-M375*, while having no significant effect on the frequency of *Arg⁺* recombinants from two pairs of *arg1* alleles. The coupling of specific *ade6* and *arg1* alleles also had no effect upon the frequency of recombinants recovered.

We next tested whether *ade6-M26* affects intergenic recombination in any of three intervals distant from *ade6*. The intervals examined were *ura2-leu2* (chromosome I), *pro2-arg3* (opposite arm of chromosome I), and *arg5-arg4* (chromosome II). The *Ade⁺* and intergenic recombinant frequencies obtained by

TABLE 2
Test for effect of *ade6-M26* on meiotic intragenic recombination at *arg1*

Strains crossed	Relevant genotypes	Recombinant frequency ($\times 10^4$)	
		Ade ⁺	Arg ⁺
GP127 \times GP126	<i>ade6-M26 arg1-14</i> \times <i>ade6-M210 arg1-230</i>	64 (1734)	0.54 (1557)
GP129 \times GP126	<i>ade6-M375 arg1-14</i> \times <i>ade6-M210 arg1-230</i>	4.0 (536)	0.58 (1046)
GP128 \times GP126	<i>ade6-M26 arg1-2</i> \times <i>ade6-M210 arg1-230</i>	70 (860)	0.77 (1549)
GP130 \times GP126	<i>ade6-M375 arg1-2</i> \times <i>ade6-M210 arg1-230</i>	7.3 (1537)	0.76 (2243)
GP144 \times GP142	<i>ade6-M26 arg1-230</i> \times <i>ade6-M210 arg1-2</i>	29 (416)	0.52 (219)
GP143 \times GP142	<i>ade6-M375 arg1-230</i> \times <i>ade6-M210 arg1-2</i>	4.4 (254)	0.57 (270)

The numbers in parentheses below each listed recombinant frequency corresponds to the number of recombinants scored for that determination.

crossing appropriate strains are listed in Table 3. *ade6-M26* increased the Ade⁺ recombinant frequency approximately 10-fold above that of *ade6-M375* as expected, but had no significant effect on the recombinant frequency for the three intergenic regions examined. These results support the hypothesis that the *M26* mutation creates a site that acts locally to stimulate recombination at *ade6*.

Effect of *ade6-M26* homozygosity on stimulation of recombination: To determine whether an interaction between the *M26* and the wild-type sequence is required for *M26* action, we analyzed the effect of *M26* homozygosity on intragenic recombination in three-factor crosses. The appropriate strains were constructed and crossed as depicted in Table 4, and the frequency of Ade⁺ recombinants generated by intragenic recombination was determined by random spore analysis. The opal nonsense suppressor *sup9* was present in the strains so that the adenine requirement caused by *M26* was suppressed (GUTZ 1971). Adenine independence was therefore determined solely by the information at the *ade6-L52* and *ade6-M210* sites. This situation allowed a comparison of the effect of *M26* on recombination when *M26* was absent (cross 1), present in one parent (crosses 2 and 3), or present in both parents (cross 4) of an *L52* \times *M210* cross. The results of crosses 1, 2, and 3 are in good agreement with results from similar crosses previously reported (GOLDMAN 1974). Compared to cross 1, the coupling of *M26* to the *L52* allele in cross 2 resulted in an approximately 10-fold increase in the frequency of recombinants. However, when *M26* was coupled to the distal *M210* allele in cross 3, little stimulation by *M26* was observed. This result can be

explained by assuming that the majority of the recombinants resulted from co-conversion, as suggested by the results of GUTZ (1971). If the conversion tracts are continuous, co-conversion of *M26* and *M210* to wild-type information at those sites would simultaneously convert the wild-type information at the *L52* site to *L52* mutant information, resulting in a recombinant converted for all three markers. The slight increase in recombinant frequency observed in cross 3 relative to that in cross 1 might be due to a low frequency of discontinuous co-conversion of *M26* and *M210* or to a low frequency of events in which the *M26*-containing parent donates wild-type information to the *L52* mutant site in the other parent.

The result of cross 4 shows that *M26* did stimulate recombination when present in both parents and that heterozygosity was not needed to trigger *M26* action. The recombinant frequency was about ten times higher in cross 4 (*M26* present in both parents) than in cross 1 (*M26* in neither parent) and was close to the sum of the frequencies of crosses 2 and 3.

The preceding genetic results suggests that the action of *M26* is conferred by the nucleotide sequence at that position and not due to a biased correction of a mismatch between it and the wild-type sequence.

Nucleotide sequence analysis of *ade6-M26*: To begin a physical analysis of *ade6-M26* we cloned, by colony hybridization, a 3.9-kb *Bam*HI fragment from an *ade6-M26* strain into pUC19, utilizing as probe an 865-bp *Eco*RI fragment from the wild-type *ade6* gene in plasmid pSPa6. The resultant plasmid, pASP1, contains the entire *ade6-M26* locus with the exception of the extreme 5' end of the gene and the first 30 nucleotides of the coding region, as deduced from the nucleotide sequence of the wild-type *ade6* gene (kindly provided by P. SZANKASI and J. KOHLI).

We predicted that the *M26* mutation was located early in the coding region near the 5' end of the gene for the following reasons: (1) *M26* was genetically mapped near one end of the *ade6* gene (GUTZ 1971); (2) *M26* must be in the coding region since the adenine requirement caused by *ade6-M26* is opal suppressible (GUTZ 1971); and (3) although most mutations of *ade6* show intragenic complementation, *ade6-M26* is not complemented by any other *ade6* allele (GUTZ 1971). This last observation can best be explained by the *M26* mutation creating an opal nonsense codon early in the 5' coding region that causes early chain termination of the *ade6* polypeptide, resulting in a short polypeptide lacking any complementing activity. We therefore determined the nucleotide sequence near the 5' end of the *ade6* coding region in pASP1, as described in MATERIALS AND METHODS. When compared to that of the wild-type *ade6* gene, the sequence revealed a single G \rightarrow T base change that creates an opal nonsense codon in the wild-type *ade6* reading frame, as predicted

TABLE 3
Test for effect of *ade6-M26* on meiotic intergenic recombination at three distant intervals

Strains crossed	Relevant genotypes	Recombinant frequency			
		Ade ⁺ (× 10 ⁴)	Ura ⁺ Leu ⁺ (× 10 ³)	Arg ⁺ Pro ⁺ (× 10 ³)	Arg ⁺ (× 10 ³)
GP98 × GP93	<i>ade6-M26 ura2-10</i> × <i>ade6-M210 leu2-120</i>	68 (79,71,51,71) *(264,349,123,271)*	13 (15,12,12,14) *(221,147,59,268)*		
GP96 × GP93	<i>ade6-M375 ura2-10</i> × <i>ade6-M210 leu2-120</i>	8.2 (8.2,10,6.9,7.5) *(724,183,45,107)*	15 (15,13,17,15) *(135,94,110,209)*		
GP92 × GP88	<i>ade6-M26 pro2-1</i> × <i>ade6-M210 arg3-124</i>	53 (62,43,54,54) *(214,163,137,147)*		76 (61,81,89,71) *(422,310,227,201)*	
GP90 × GP88	<i>ade6-M375 pro2-1</i> × <i>ade6-M210 arg3-124</i>	5.8 (6.9,5.7,4.3,6.1) *(528,151,117,303)*		67 (63,93,52,61) *(508,230,146,147)*	
GP155 × GP99	<i>ade6-M26 arg5-189</i> × <i>ade6-M210 arg4-55</i>	36 (23,48) *(185,163)*			28 (25,31) *(209,104)*
GP153 × GP99	<i>ade6-M375 arg5-189</i> × <i>ade6-M210 arg4-55</i>	5.6 (5.4,5.7) *(177,175)*			24 (23,25) *(153,148)*

The recombinant frequency listed for each cross corresponds to the mean of four frequencies which are listed in the adjacent parentheses. These four frequencies were obtained by performing each cross twice and plating each cross mixture on two separate occasions. Only one experimental cross with two platings was performed for GP155 × GP99 and GP153 × GP99. The numbers listed in starred parentheses are the number of recombinants scored for determining the recombinant frequency in parentheses above them.

TABLE 4
Effect of *ade6-M26* homozygosity on *ade6* meiotic intragenic recombination

Cross No.	Strains	<i>ade6</i> markers	Ade ⁺ recombinant frequency (× 10 ⁴)	
1	GP26 × GP27	+ <i>L52</i> + <i>sup9</i> + + <i>M210</i> <i>sup9</i>	1.5	(1.2,2.1,0.9,1.8) *(36,21,6,8)*
2	GP28 × GP27	<i>M26</i> <i>L52</i> + <i>sup9</i> + + <i>M210</i> <i>sup9</i>	13	(13.3,14.6,11.4,11.1) *(180,168,106,60)*
3	GP26 × GP29	+ <i>L52</i> + <i>sup9</i> <i>M26</i> + <i>M210</i> <i>sup9</i>	2.6	(2.2,2.4,3.0,2.8) *(24,40,18,19)*
4	GP28 × GP29	<i>M26</i> <i>L52</i> + <i>sup9</i> <i>M26</i> + <i>M210</i> <i>sup9</i>	17	(14,21,20,13) *(66,130,82,58)*

The recombinant frequency listed for each cross is the mean of four frequencies which are listed in the adjacent parentheses. The four frequencies for each cross were obtained from four separate experimental crosses. The numbers in starred parentheses are the number of recombinants scored in each experiment. Recombinants growing on MMA from cross 4 are of the genotype *ade6-M26 sup9*; such spores germinate and form colonies on MMA only 50% as efficiently as *ade6⁺ sup9* spores (our unpublished data). The frequencies reported for cross 4 represent the observed frequency multiplied by 2 to correct for the decreased plating efficiency of these spores.

(Figure 3). No other changes were observed in the 110 bp to the left and 140 bp to the right of *M26* that were sequenced. This single base-pair change has been confirmed by sequence analysis of an independent *M26* clone, *pade6-M26* (P. SZANKASI, W.-D. HEYER, and J. KOHLI, personal communication). This clone is identical in structure to *pade6-L469* (Figure 2), except it contains the *M26* allele rather than *L469*. The region of *pade6-M26* sequenced was the 815 bp *BamHI-HindIII* fragment, which extends 110 bp to the left and 715 bp to the right of *M26*.

To verify that the G → T base change creating the opal codon is also responsible for the recombination hotspot activity, we transferred the opal mutation from the 815 bp *BamHI-HindIII* fragment of *pade6-M26* (sequenced by P. SZANKASI *et al.*) to the chro-

mosomal *ade6* gene (see MATERIALS AND METHODS). Each of two Ade⁻ transformants was opal-suppressible for the adenine requirement, and in crosses with the *ade6-M210* allele displayed the high frequency of recombinants characteristic of *M26* strains (data not shown).

Meiosis-specific action of *ade6-M26*: To determine whether *ade6-M26* stimulates mitotic as well as meiotic recombination, we constructed strains to test the mitotic and meiotic effects of *ade6-M26* on two types of recombination events: interchromosomal recombination in heteroallelic diploids and recombination between the chromosome and a multicopy plasmid containing the *ade6-L469* heteroallele. In each case the presence of *ade6-M26* increased the meiotic recombinant frequency 9–14-fold relative to

ade6-M210 and *ade6-M26* × *ade6-M26-M210*. Although it was concluded that *M26* does act in the homozygous state, the conclusion was based on a very small number of revertant tetrads. In addition, the control of determining the conversion frequency of the *M210* allele in the absence of *M26* was not reported. We therefore performed our random spore analysis of *ade6* intragenic recombination to confirm that *M26* stimulates recombination when homozygous and that the action is due to the nucleotide sequence at *M26* and not due to a correction bias of a mismatch formed at that position.

Mutations with properties similar to *M26* have been reported in other organisms. The *YS17* mutation in the *buff* locus of *Sordaria brevicollis* (MACDONALD and WHITEHOUSE 1979), and the *cog*⁺ allele of *Neurospora crassa* (ANGEL, AUSTIN and CATCHESIDE 1970) have also been postulated to create fungal recombination initiation sites. For both *cog* and *YS17*, unlinked genes affecting the recombination promoted by these sites have been reported (ANGEL, AUSTIN and CATCHESIDE 1970; MACDONALD and WHITEHOUSE 1982). Presumably, these unlinked genes encode products that directly interact with the sites or control the expression of others that do. The isolation of gene products that interact with the *M26* site, the determination of their biochemical activities, and the determination of the primary biochemical event that takes place at or near *M26* will lead to a better understanding of both the regulation and the mechanisms involved in homologous recombination.

We especially thank PHILIPPE SZANKASI, WOLF-DIETRICH HEYER, and JURG KOHLI for generously sharing plasmids, strains and nucleotide sequences before publication. We also thank STEPHEN GOLDMAN, ANWAR NASIM, HANSPETER AMSTUTZ and GINGER ZAKIAN for strains, MARK RIXON and RICH GELINAS for sequencing advice and materials, and GARY BRAEDT, SUSAN HOLBECK, KAREN LARSON and ANDREW TAYLOR for helpful comments on the manuscript. This work was supported by the National Institutes of Health grant GM31693 to G.R.S. A.S.P. is the recipient of a Ginsberg predoctoral fellowship from the Fred Hutchinson Cancer Research Center.

LITERATURE CITED

- ADONDI, G., and H. HESLOT, 1970 Etude des conversions mitotiques au niveau du gene *ad-9* de *Schizosaccharomyces pombe*. *Mutat. Res.* **9**: 41–58.
- ANGEL, T., B. AUSTIN and D. G. CATCHESIDE, 1970 Regulation of recombination at the *his3* locus in *Neurospora crassa*. *Aust. J. Biol. Sci.* **23**: 1229–1240.
- 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., M. PIPER and P. NURSE, 1982 Construction of a *Schizosaccharomyces pombe* gene bank in a yeast bacterial shuttle vector and its use to isolate genes by complementation. *Mol. Gen. Genet.* **187**: 326–329.
- CHEN, E. Y., and P. H. SEEBURG, 1985 Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. *DNA* **4**: 165–170.
- FLORES DA CUNHA, M., 1970 Mitotic mapping of *Schizosaccharomyces pombe*. *Genet. Res.* **16**: 127–144.
- GOLDMAN, S. L., 1974 Studies on the mechanism of induction of site specific recombination in the *ade6* locus of *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* **132**: 347–361.
- GOLDMAN, S. L., and S. SMALLETS, 1979 Site specific induction of gene conversion: the effects of homozygosity of the *ade6* mutant *M26* of *Schizosaccharomyces pombe* on meiotic gene conversion. *Mol. Gen. Genet.* **173**: 221–225.
- GUTZ, H., 1963 Untersuchungen zur feinstruktur der gene *ade7* und *ade6* von *Schizosaccharomyces pombe* Lind. Habilitationsschrift, Technische Universtat Berlin.
- GUTZ, H., 1971 Site specific induction of gene conversion in *Schizosaccharomyces pombe*. *Genetics* **69**: 317–337.
- GUTZ, H., H. HESLOT, U. LEUPOLD and N. LOPRIENO, 1974 *Schizosaccharomyces pombe*. pp. 395–446. In: *Handbook of Genetics*, Vol. 1, Edited by R. C. King. Plenum Press, New York.
- HANAHAN, D., 1983 Studies on transformation of *E. coli* with plasmids. *J. Mol. Biol.* **166**: 557–580.
- HANAHAN, D., and M. MESELSON, 1980 Plasmid screening at high colony density. *Gene* **10**: 63–67.
- HEYER, W.-D., M. SIPICZKI and J. KOHLI, 1986 Replicating plasmids in *Schizosaccharomyces pombe*: improvement of symmetric segregation by a new genetic element. *Mol. Cell. Biol.* **6**: 80–89.
- HOLLIDAY, R., 1964 A mechanism for gene conversion in fungi. *Genet. Res.* **5**: 282–304.
- KOHLI, J., H. HOTTINGER, P. MUNZ, A. STRAUSS and P. THURIAUX, 1977 Genetic mapping in *Schizosaccharomyces pombe* by mitotic and meiotic analysis and induced haploidization. *Genetics* **87**: 471–489.
- LOSSON, R., and F. LACROUTE, 1983 Plasmids carrying the yeast OMP decarboxylase structural and regulatory genes: transcription regulation in a foreign environment. *Cell* **32**: 371–377.
- MACDONALD, M., and H. WHITEHOUSE, 1979 A *buff* spore color mutant in *Sordaria brevicollis* showing high frequency conversion. 1. Characteristics of the mutant. *Genet. Res.* **34**: 87–119.
- MACDONALD, M., and H. WHITEHOUSE, 1982 A *buff* spore color mutant in *Sordaria brevicollis* showing high frequency conversion. 2. Loss of the high frequency conversion. *Genet. Res.* **41**: 155–163.
- MESELSON, M., and C. RADDING, 1975 A general model for genetic recombination. *Proc. Natl. Acad. Sci. USA* **75**: 358–361.
- NURSE, P., 1975 Genetic control of cell size at cell division in yeast. *Nature* **256**: 547–551.
- SCHUCHERT, P., and J. KOHLI, 1988 The *ade6-M26* mutation of *Schizosaccharomyces pombe* increases the frequency of crossing over. *Genetics* **119**: 507–515.
- SMITH, G. R., S. M. KUNES, D. W. SCHULTZ, A. F. TAYLOR and K. TRIMAN, 1981 Structure of Chi hotspots of generalized recombination. *Cell* **24**: 429–436.
- SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-break repair model for recombination. *Cell* **33**: 25–35.
- WHITEHOUSE, H. L. K., 1982 *Genetic Recombination: Understanding the Mechanisms*. John Wiley & Sons, New York.
- YANISCH-PERRON, C., J. VIEIRA and J. MESSING, 1985 Improved *M13* phage cloning vectors and host strains: nucleotide sequences of the *M13mp18* and *pUC19* vectors. *Gene* **33**: 103–119.