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

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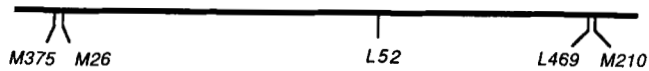
FIGURE 1.—Genetic map of the *ade6* locus showing the approximate locations of the mutations used in this work.

TABLE 1

S. pombe strains

SMITH laboratory strain	Genotype	Source or derivation
GP3	h^- *ade6-M26 sup9*	S. GOLDMAN
GP4	h^+ *ade6-M210*	S. GOLDMAN
GP6	h^+ *ade6-M375*	S. GOLDMAN
GP8	h^- *ade6-M26-M210*	S. GOLDMAN
GP14	h^+ *ade6-L52*	S. GOLDMAN
GP16	h^+ *ade6-M26-L52*	S. GOLDMAN
GP17	h^+ *ade6-M26-M210*	GP8 × GP19
GP19	h^+	S. GOLDMAN
GP23	h^- *ade6-M26*	S. GOLDMAN
GP24	h^+ *ade6-M26*	GP3 × GP19
GP25	h^- *sup9*	GP3 × GP19
GP26	h^- *ade6-L52 sup9*	GP14 × GP25
GP27	h^+ *ade6-M210 sup9*	GP4 × GP25
GP28	h^- *ade6-M26-L52 sup9*	GP16 × GP25
GP29	h^+ *ade6-M26-M210 sup9*	GP17 × GP25
GP31	h^- *ura4-294*	V. ZAKIAN
GP42	h^+ *ade6-M210 ura4-294*	GP4 × GP31
GP51	h^{90} *ade6-M26*	GP24
GP52	h^{90} *ade6-M375*	GP6
GP57	h^{90} *ade6-M26 ura4-294*	GP31 × GP51
GP58	h^{90} *ade6-M375 ura4-294*	GP31 × GP52
GP66	h^{90} *ade6-M26* (pade6-L469)	GP57 + pade6-L469
GP67	h^{90} *ade6-M375* (pade6-L469)	GP58 + pade6-L469
GP68	h^+ *ade6-M210 ura4-294* / h^+ *ade6-M26 arg1-2*	GP42 × GP128
GP69	h^- *ade6-M210 ura4-294* / h^- *ade6-M375 arg1-2*	GP42 × GP130
GP73	h^- *pro2-1*	J. KOHLI
GP75	h^- *ura2-10*	J. KOHLI
GP77	h^- *leu2-120*	J. KOHLI
GP83	h^- *arg4-55*	J. KOHLI
GP85	h^- *arg5-189*	J. KOHLI
GP88	h^+ *ade6-M210 arg3-124*	J. KOHLI
GP90	h^- *ade6-M375 pro2-1*	GP6 × GP73
GP92	h^- *ade6-M26 pro2-1*	GP24 × GP73
GP93	h^+ *ade6-M210 leu2-120*	GP4 × GP77
GP96	h^- *ade6-M375 ura2-10*	GP6 × GP75
GP98	h^- *ade6-M26 ura2-10*	GP24 × GP75
GP99	h^+ *ade6-M210 arg4-55*	GP4 × GP83
GP102	h^- *arg1-14*	A. NASIM
GP103	h^- *arg1-2*	A. NASIM
GP105	h^- *arg1-230*	H. AMSTUTZ
GP126	h^+ *ade6-M210 arg1-230*	GP4 × GP105
GP127	h^- *ade6-M26 arg1-14*	GP24 × GP102
GP128	h^- *ade6-M26 arg1-2*	GP24 × GP103
GP129	h^- *ade6-M375 arg1-14*	GP6 × GP102
GP130	h^- *ade6-M375 arg1-2*	GP6 × GP103
GP142	h^+ *ade6-M210 arg1-2*	GP4 × GP103
GP143	h^- *ade6-M375 arg1-230*	GP6 × GP105
GP144	h^- *ade6-M26 arg1-230*	GP24 × GP105
GP153	h^- *ade6-M375 arg5-189*	GP6 × GP85
GP155	h^- *ade6-M26 arg5-189*	GP24 × GP85

Eppendorf microcentrifuge, resuspended with 10 μl 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 μg/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 to 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 3×10^8 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 h^{90} 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-straining (sporulating) h^{90} revertants.

Crosses to test for localized *ade6-M26* action: Standard crosses were performed on MEA slants and spores plated onto appropriately supplemented (50–100 μg/ml) or nonsupplemented 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 *Sau*3A 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, SIPICZKI 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.

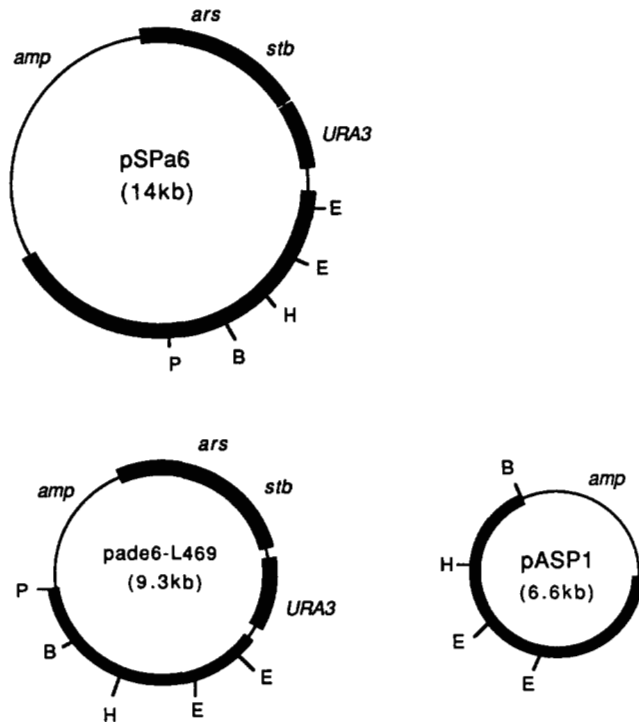**Cloning and sequence analysis of *ade6-M26*:** *S. pombe*

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, SIPICZKI 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, *Hin*dIII; P, *Pvu*II. Transcription of *ade6* initiates approximately 90 bp upstream of the *Bam*HI site and proceeds toward the *Hin*dIII 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 µl) prepared from strain GP18 (*h⁻ leu1-32*) were co-transformed with 1 µg each of the 815 bp *Bam*HI-*Hin*dIII 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 µg/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 × 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 × GP126	ade6-M26 arg1-14 × ade6-M210 arg1-230	64 (1734)	0.54 (1557)
GP129 × GP126	ade6-M375 arg1-14 × ade6-M210 arg1-230	4.0 (536)	0.58 (1046)
GP128 × GP126	ade6-M26 arg1-2 × ade6-M210 arg1-230	70 (860)	0.77 (1549)
GP130 × GP126	ade6-M375 arg1-2 × ade6-M210 arg1-230	7.3 (1537)	0.76 (2243)
GP144 × GP142	ade6-M26 arg1-230 × ade6-M210 arg1-2	29 (416)	0.52 (219)
GP143 × GP142	ade6-M375 arg1-230 × ade6-M210 arg1-2	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 × 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 BamHI fragment from an ade6-M26 strain into pUC19, utilizing as probe an 865-bp EcoRI 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 → 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⁺ (× 10⁴)	Ura⁺Leu⁺ (× 10³)	Arg⁺Pro⁺ (× 10³)	Arg⁺ (× 10³)
GP98 × GP93	*ade6-M26 ura2-10* × *ade6-M210 leu2-120*	68 (79,71,51,71) *(264,349,123,271)*	13 (15,12,12,14) *(221,147,59,268)*		
GP96 × GP93	*ade6-M375 ura2-10* × *ade6-M210 leu2-120*	8.2 (8.2,10,6.9,7.5) *(724,183,45,107)*	15 (15,13,17,15) *(135,94,110,209)*		
GP92 × GP88	*ade6-M26 pro2-1* × *ade6-M210 arg3-124*	53 (62,43,54,54) *(214,163,137,147)*		76 (61,81,89,71) *(422,310,227,201)*	
GP90 × GP88	*ade6-M375 pro2-1* × *ade6-M210 arg3-124*	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	*ade6-M26 arg5-189* × *ade6-M210 arg4-55*	36 (23,48) *(185,163)*			28 (25,31) *(209,104)*
GP153 × GP99	*ade6-M375 arg5-189* × *ade6-M210 arg4-55*	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	*ade6* markers				Ade⁺ recombinant frequency (× 10⁴)
1	GP26 × GP27	+ L52 + sup9 / + + M210 sup9				1.5 (1.2,2.1,0.9,1.8) *(36,21,6,8)*
2	GP28 × GP27	M26 L52 + sup9 / + + M210 sup9				13 (13.3,14.6,11.4,11.1) *(180,168,106,60)*
3	GP26 × GP29	+ L52 + sup9 / M26 + M210 sup9				2.6 (2.2,2.4,3.0,2.8) *(24,40,18,19)*
4	GP28 × GP29	M26 L52 + sup9 / M26 + M210 sup9				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 *Bam*HI-*Hind*III 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 chromosomal *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

TABLE 5

Comparison of the effect of *ade6-M26* on meiotic *vs.* mitotic recombination

Type of recombination event	Mitotic frequency Ade$^+$ cells ($\times$ 10^4)		Ratio M26/M375	Meiotic frequency Ade$^+$ spores ($\times$ 10^4)		Ratio M26/M375
Chromosome $\times$ chromosome						
ade6-M26 $\times$ *ade6-M210*	0.73	(0.65–0.84)		53	(29–70)	
ade6-M375 $\times$ *ade6-M210*	0.84	(0.62–1.1)	0.9	5.9	(4.0–8.2)	9.0
Chromosome $\times$ multicopy plasmid						
ade6-M26 $\times$ *pade6-L469*	3.5	(3.2–12.3)		140	(120–180)	
ade6-M375 $\times$ *pade6-L469*	3.5	(3.2–5.4)	1.0	10	(10–14)	14

Mitotic frequencies were determined by fluctuation analysis of strains GP69 and GP68 for chromosome $\times$ chromosome events (five cultures each) and strains GP67 and GP66 for chromosome $\times$ plasmid events (seven cultures each). The frequencies listed are those of the cultures with the median recombinant frequency and the range is listed in parentheses. The meiotic frequencies for the chromosome $\times$ chromosome events were obtained from the crosses listed in Tables 2 and 3. Listed is the mean of six crosses with the range listed in parentheses. The meiotic frequencies for chromosome $\times$ plasmid events were obtained as described in MATERIALS AND METHODS. The frequencies listed correspond to the median of seven matings and the range listed in parentheses.

wild-type: 5'- ATT GAT GGA GGA CGT GAG -3'

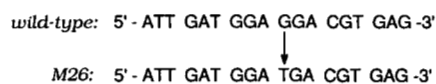M26: 5'- ATT GAT GGA TGA CGT GAG -3'

FIGURE 3.—Comparison of wild-type and *ade6-M26* nucleotide sequences. The nucleotides are presented in blocks of three representing the reading frame of the wild-type gene, determined by P. SZANKASI, W.-D. HEYER and J. KOHLI (personal communication).

the *ade6-M375* control, while having no significant effect on the mitotic recombinant frequency (Table 5).

From these results we conclude that (1) *ade6-M26* appears to stimulate only meiotic recombination and (2) *ade6-M26* can exhibit its marker effect by recombining with a multicopy plasmid during meiosis.

DISCUSSION

To account for the behavior of *M26*, GUTZ (1971) and GOLDMAN (1974) hypothesized that the mutation creates a site that undergoes preferential single-strand DNA breakage, followed by exonucleolytic degradation towards both ends of the *ade6* gene. The chromatid undergoing degradation then acts as a recipient of genetic information, using an intact homologous chromatid as a donor template. This can account for the high conversion frequency of *M26*, co-conversion of coupled alleles, and the observed disparity. This hypothesis suggests that *M26* stimulates recombination by acting as an initiation site in recombination. Our results reported here are consistent with this view.

Nucleotide sequence analysis showed *M26* to be a single G → T base-pair change. Single base-pair changes both create and inactivate Chi recombination hotspots in *E. coli* and phage lambda (SMITH *et al.* 1981). As predicted by the hypothesis that the *M26* mutation creates a site enhancing meiotic recombination, recombination was enhanced apparently only in the vicinity of *M26* and only during meiosis. Using

heteroallelic duplications of *ade6*, SCHUCHERT and KOHLI (1988) have observed that *M26* stimulates the frequency of crossovers but does so only during meiosis. The meiosis-specific action of *M26* may be due to the meiosis-specific expression of a gene product that interacts with the *M26* site, or due to a difference in the meiotic chromatin structure around *M26* relative to that during mitotic growth, allowing differential accessibility to recombination-promoting enzymes.

Current models for fungal recombination generally suggest that gene conversion is due either to the formation of heteroduplex DNA followed by the repair of mismatched bases (HOLLIDAY 1964; MESELSON and RADDING 1975), or due to the repair of a double-strand gapped chromatid (SZOSTAK *et al.* 1983). *M26* might act to stimulate the formation of heteroduplex DNA by being a site for preferential DNA breakage. Alternatively, *M26* could act at the level of mismatch correction. For example, when *ade6* alleles other than *M26* are contained within a region of hybrid DNA, resultant mismatches might be efficiently repaired (as shown by the low frequency of postmeiotic segregation, GUTZ 1971) and corrected nearly always in a restorative fashion to generate 2 + :2 − tetrads. In contrast, the sequence including the *M26* mutation might be such that when this allele is contained in a region of heteroduplex with wild-type sequence, the *M26*-containing strand is preferentially excised and corrected in a way to yield an elevated frequency of 3 + :1 *M26* conversion tetrads. Such a mechanism could also account for bidirectional co-conversion.

GOLDMAN and SMALLETS (1979) first addressed the possibility that *M26* acts at the level of mismatch correction by asking whether *M26* acts when homozygous in a cross, where there is no possibility for a mismatch at the *M26* site. GOLDMAN and SMALLETS analyzed meiotic tetrads to monitor the conversion of the *ade6-M210* allele in the crosses *ade6-M26* $\times$

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 convertant 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.

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