SITE SPECIFIC INDUCTION OF GENE CONVERSION IN *SCHIZOSACCHAROMYCES POMBE*[1]

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Manuscript received December 17, 1969
Revised copy received August 17, 1971

IT is today a well established fact that, in addition to classical crossing over which is reciprocal, nonreciprocal recombination or gene conversion also occurs. Hypotheses on gene conversion have been published by WHITEHOUSE (1963, 1965), HOLLIDAY (1964, 1968), WHITEHOUSE and HASTINGS (1965), TAYLOR (1967), STAHL (1969) and PASZEWSKI (1970); for reviews see EMERSON (1969) and WHITEHOUSE (1970).

The work reported in this paper is concerned with the conversion behavior of the *ade*6 mutant M26 of the fission yeast *Schizosaccharomyces pombe* Lindner. The mutant M26 is exceptional by giving approximately 13 times more prototrophic recombinants in intragenic crosses than "normal" *ade*6 mutants (GUTZ 1963). No other mutant with a behavior similar to M26 was found among 394 *ade*6 and 263 *ade*7 mutants of independent origin of *S. pombe* (LEUPOLD 1961; GUTZ 1961, 1963; LEUPOLD and GUTZ 1965). In the present paper the investigation of M26 is extended to tetrad analyses from crosses of M26 with the wild type and with nonidentical *ade*6 alleles. About 3 to 5% aberrant tetrads were obtained; the mutation site of M26 was converted predominantly to the wild-type site. In crosses heterozygous for two or three sites within the *ade*6 gene preferential symmetrical or polarized conversion occurred frequently. M26 shows a pronounced marker effect with respect to intragenic recombination and gene conversion. Alleles mapping close to M26 neither yield as many prototrophs as M26 if crossed with nonidentical alleles, nor do they give as many aberrant tetrads if crossed with the wild type. Part of the results have been previously reported in abstracts (GUTZ 1966, 1968).

MATERIALS AND METHODS

Life cycle: The normal life cycle of *S. pombe* is that of a haplontic organism (LEUPOLD 1958, 1970). The diplophase is confined to the zygote, which is formed by pairwise copulation of haploid vegetative cells of compatible mating types. Copulation occurs at the end of vegetative growth and is preceded by a strong sexual agglutination of the cells. The zygotes immediately undergo meiosis and spore formation. Four spores are formed in each zygote; the spores, if transferred to a fresh medium, germinate into haploid vegetative cells. In the present work, heterothallic strains of the mating types h^{+N} and h^{-} were used (see LEUPOLD 1958). h^{+N} is the standard + mating type used in genetic work with *S. pombe*, and the abbreviated symbol h^{+} will be used in this paper.

[1] Supported by NSF Grant GB-15148 and NIH Grant GM 13234.

Strains: Mutants of the *ade*6 gene require adenine for growth; they are blocked physiologically between the AIR and CAIR steps of adenine biosynthesis (FISHER 1969) and accumulate a red pigment if grown on media with limited amounts of adenine. The fine structure of this gene has been studied extensively (LEUPOLD 1961; GUTZ 1963; LEUPOLD and GUTZ 1965). Most *ade*6 mutations show complementation if combined with appropriate nonidentical alleles in diploid cells.

The mutant M26 was induced with 180 keV X rays at a dose of 30,000 r (322 r/min; GUTZ 1963). M26 does not complement with any other *ade*6 mutant. Nineteen nonidentical *ade*6 mutants were used for crosses with M26. The numbers of these mutants will be given with the pertinent experiments (Figure 1 and Table 1). Mutants with an L before their numbers had been induced with ultraviolet light by LEUPOLD (1961, and personal communication), whereas the mutants marked with an M (with the exception of M26) had been induced with nitrous acid (GUTZ 1963). (Note: The strains obtained from LEUPOLD were cited in GUTZ 1963 without an L before their collection numbers.) All mutants used (including M26) map at single sites in the *ade*6 locus (GUTZ 1963; LEUPOLD and GUTZ 1965), and they are known to give no, or only very few (less than 10^{-6}), spontaneous reversions.

The *ade*6 mutations had been induced in the wild-type strain L975 which has the mating type $h+$. By crossing the *ade*6 mutants with the wild-type strain L972 (mating type h^-), h^- strains were isolated carrying the corresponding alleles. For simplicity these h^- strains have the same collection numbers as the original mutants, but, when necessary, the mating types are given in brackets behind the numbers for distinction.

In the crosses done for tetrad analyses strains with an additional auxotrophic marker (uracil requirement) were used:

SG63: h^- *ADE6 ura*1
SG140: h^- *ade*6-L52 *ura*1
SG141: $h+$ *ade*6-L52 *ura*1
SG146: h^- *ade*6-M216 *ura*1
SG147: $h+$ *ade*6-M216 *ura*1

The *ura*1 marker and the mating-type alleles were used to check whether or not tetrads with an aberrant *ade*6 segregation show a normal segregation for both these genes. These three loci are not linked; outside markers are not known for the *ade*6 locus.

In addition to the above strains, some spore cultures from aberrant tetrads were also used for crosses:

A301b: $h+$ *ADE6 ura*1
A801a: $h+$ *ade*6-M216 *URA*1
A801b: h^- *ade*6-M26 *URA*1
A801c: $h+$ *ade*6-M216 *ura*1
A802b: $h+$ *ade*6-M26 *URA*1
A814a: $h+$ *ade*6-M26 *URA*1
A814d: h^- *ade*6-M216 *ura*1
A505a: h^- *ade*6-M26-L52 *ura*1

A301b is from an aberrant tetrad 3 + : 1 M26 of cross M26 ($h+$) × SG63, and A801a to A814d are from aberrant tetrads 3 M216+ : 1 +M26 of cross M26 ($h+$) × SG146. A505a has mutations at two *ade*6 sites; it originated in cross M26 ($h+$) × SG141 (see Tables 2a and 2b).

The gene symbols are given in accordance with the recommendations of the Yeast Genetics Conference in Chalk River, Canada, October 1970. Wild-type alleles are symbolized by capital letters. The different *ade*6 heteroalleles are indicated by numbers after the gene symbol; these numbers are identical with the numbers under which the mutants are kept in our strain collection.

Media and incubation temperatures: Yeast-extract agar (YEA; 0.5% Bacto-yeast extract, 3% glucose, 2% agar), malt-extract agar (MEA; 3% Bacto-malt extract, 2% agar, supplemented with 75 μg adenine/ml), minimal agar (MMA: LEUPOLD 1955a), and synthetic sporulation agar (SPA; ANGEHRN 1964, supplemented with 20 μg adenine/ml). On YEA, *ade*6 colonies show the red pigment very distinctly. MMA with and without appropriate supplements was used to check the requirements of the single spore cultures. All slants and plates on which vegetative growth was desired were incubated at 30°C; the MEA slants with the crosses and the MEA plates for the mating-type tests were incubated at 25°C.

Crosses: The parent strains needed for the crosses were always first streaked on YEA, and a single nonsporulating colony of each strain was inoculated on a YEA slant supplemented with

75 μg adenine/ml. After two days the strains were mass-mated on MEA slants. The isolation of single colonies was done to avoid homothallic mutants (LEUPOLD 1958). This procedure also ensured that strains were not used which might have had accumulated diploid cells, spontaneous reversions to prototrophy (e.g., due to suppressors), or spontaneous mutations in genes involved in steps prior to *ade*6 in adenine biosynthesis (such digenic mutants no longer form the red pigment on YEA; LEUPOLD 1955a).—The crosses of MEA slants contained many asci after two days. The nine different types of crosses done for tetrad analysis have been given running numbers throughout Tables 2 to 4.

Tetrad analyses: Asci from 2 or 3 day old crosses were used. We performed the tetrad analyses with the help of de Fonbrune micromanipulators in the following way: single asci were isolated on thin layers (HAEFNER 1967) of SPA, the layers were then kept for 17 to 20 hr at room temperature (22–25°C). After this time the walls of about 80% of the asci were dissolved. We separated the spores by micromanipulation and transferred the SPA layers to YEA plates which were incubated 3 to 4 days at 30°C. The plates were kept for at least an additional 24 hr at room temperature; this step was important for the evaluation of the layers, as at room temperature the red colors of adenine-requiring spore colonies became very distinct.

Recombination analyses with free ascospores: The MEA slants with the crosses were incubated 7 days at 25°C, and were then kept 7 more days in the refrigerator (about 3°C). After this time the walls of all asci have dissolved spontaneously. We treated the mixtures of free ascospores and vegetative cells for 30 min with 30% alcohol at 18°C (LEUPOLD 1957). The alcohol kills only the vegetative cells; therefore, by plating on appropriate media, one obtains only colonies originating from spores. This technique does not result in a selective elimination of *ade*6 versus *ADE*6 spores. Before the colonies were counted, the plates were treated with iodine vapors to detect sporulating colonies (LEUPOLD 1955b). This procedure is routinely used in the work with *S. pombe;* it makes it possible to exclude rare crosses which contain significant numbers of homothallic or diploid spores. The iodine treatment was used also in all other experiments whenever its application was pertinent (e.g., in the mating-type tests, the allele tests, and the tests for suppressors).

Allele tests: They were performed by crossing cultures obtained from red spore colonies with appropriate single site mutants. From these crosses about 5×10^6 spores were streaked on MMA + uracil (GUTZ 1963). If the strains are homoallelic, no (or occasionally very few, e.g. one to five) colonies develop. In the present experiments, heteroallelism was always indicated by many *ADE*6 colonies (depending on the alleles involved, about a hundred to several thousand) because all mutants used for tetrad analyses give more than 60 prototrophs per 10^6 spores if crossed with each other. Thus the allele tests gave always an unambiguous result.

Suppressor tests: Cultures from white spore colonies (adenine independent) were tested for the possible presence of suppressors by crossing them with the wild-type strains L975 or L972, respectively. After alcohol treatment, spores were streaked on YEA. If a suppressor is present, some red colonies would develop on the plates.

Other experimental techniques: They have been described elsewhere (LEUPOLD 1955a, b; GUTZ 1963; ANGEHRN 1964; TREICHLER 1964). LEUPOLD (1970) has reviewed the genetical methods used with *S. pombe* as well as the major results obtained with this yeast.—The 95% confidence limits shown in Tables 2a and 3 were taken from Tables 8 and 9a in WEBER (1957).

EXPERIMENTS AND RESULTS

I. *Frequencies of prototrophic recombinants in intragenic crosses:* M26 yields an unusually high number of prototrophic recombinants in crosses with nonidentical alleles (GUTZ 1963). To extend our previous data, M26 was crossed with a total of 19 nonidentical *ade*6 mutants. The mutants were chosen from all parts of the *ade*6 map (Figure 1). Each cross was done several times. We plated free ascospores of the crosses in appropriate dilutions on MMA and MMA sup-

TABLE 1

Frequencies of prototrophic spores in crosses of ade6-*M26 and* ade6-*M375 with nonidentical* ade6 *alleles (plating of free ascospores)*

Crossed with (*ade6* mutants of mating type h^+ or h^-)	M26					M375					
			Number of colonies on					Number of colonies on			
	Number of crosses*	Factor (F)†	MMA	MMA + adenine	Frequency of prototrophs $\times 10^6$ (R)‡	Number of crosses*	Factor (F)†	MMA	MMA + adenine	Frequency of prototrophs $\times 10^6$ (R)‡	Increase§
L406	3	10^4	1,346	817	165	3	10^4	228	880	25.9	6.4
L250	3	10^4	954	1,033	92.4	3*	10^4	134	748	17.9	5.2
M216	3*	10^4	659	969	68.0	3*	10^5	613	323	19.0	3.6
M375	3*	10^5	78	888	0.9	2*	10^5	0	590	0.0	—
M26	5*	10^5	1	1,205	0.0	3*	10^5	78	888	0.9	—
L555	3	10^5	854	679	12.6	3	10^5	198	486	4.1	3.1
L415	3	10^4	463	923	50.2	3	10^5	490	372	13.2	3.8
L687	3	5×10^3	1,452	1,151	252	3*	10^4	257	526	48.9	5.2
L428	3	5×10^2	139	927	300	3	10^4	289	532	54.3	5.5
L743	3*	2×10^3	1,413	942	750	3*	10^4	544	621	87.6	8.6
L463	4*	5×10^2	938	964	1,940	3*	10^4	991	553	179	10.8
L616	5	5×10^2	2,499	1,520	3,290	5	5×10^3	1,492	1,933	154	21.4
L52	5	10^2	1,127	3,112	3,620	5	10^4	2,380	757	314	11.5
L539	3	2.5×10^2	941	780	4,830	3	5×10^3	705	355	397	12.2
L615	3	2×10^2	690	544	6,340	3	5×10^3	653	369	354	17.9
L149	3	10^2	411	622	6,610	3*	5×10^3	1,356	552	491	13.5
L702	2	10^2	656	1,955	3,360	2	2×10^3	1,059	1,049	504	6.7
M210	6*	10^2	1,553	2,678	5,800	2	2×10^3	1,220	1,134	538	10.8
L432	5	10^2	1,376	1,868	7,370	3	5×10^3	1,445	514	562	13.1
L469	3	10^2	596	646	9,230	5	2×10^3	1,279	889	719	12.8

* If the number of crosses is marked (*), both M26(h^+) and M26(h^-) or M375(h^+) and M375(h^-) were used for the crosses; otherwise only the h^+ strains were used.

$$\dagger F = \frac{\text{Number of spores plated on MMA}}{\text{Number of spores plated on MMA} + \text{adenine}}$$

$$\ddagger R = \frac{(\text{Number of colonies on MMA}) \times 10^6}{(\text{Number of colonies on MMA} + \text{adenine}) \times F}$$

§ R Values of the crosses with M26 divided by R values of the corresponding crosses with M375.

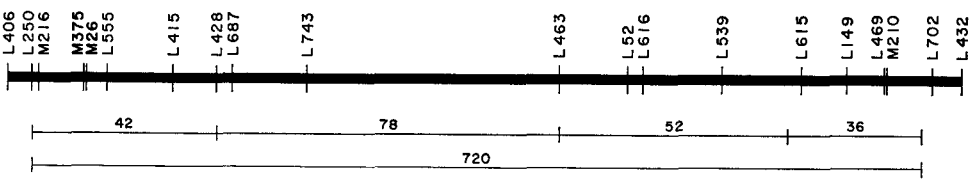


FIGURE 1.—Abbreviated fine structure map of the *ade*6 locus of *S. pombe* showing the approximate location of the mutants used in the present experiments. Below the map some distances are given as numbers of prototrophic recombinants per 10^6 ascospores (redrawn from GUTZ 1963 and LEUPOLD and GUTZ 1965). In the *ade*6 locus a pronounced map expansion (HOLLIDAY 1968) is observed.

plemented with 20 μg adenine/ml. In general three MMA and MMA + adenine plates were used in a single experiment, the minimal medium receiving 10^2 to 10^5 times more spores than the supplemented medium (see factors in Table 1). If possible, we diluted the spore suspensions so that 70–120 colonies could be expected per plate. The results are listed in Table 1.[2] To compile Table 1 the colony numbers of the individual crosses of each type of cross have been totaled. The recombination values shown are therefore weighted mean values; they are given as numbers of prototrophic spores per 10^6 ascospores.

M26 is not homoallelic with any other known *ade*6 mutant, but its mutation site maps very close to the site of M375 (0.9 prototrophs per 10^6 spores). M375 is located near the left end of the *ade*6 map. The largest numbers of prototrophs given by M26 are as many as 12 to 13 times higher than those obtained normally in the *ade*6 locus. The most distant "normal" *ade*6 mutants yield approximately 720 prototrophs per 10^6 spores if crossed with each other. As already mentioned, no other *ade*6 mutant is known which gives, in intragenic crosses, as many prototrophs as M26.

Because the mutant M375 maps very close to M26, it was chosen for control crosses. M375 was crossed with the same mutants used in the crosses with M26. The recombination frequencies obtained (Table 1) fit well into the normal recombination map of the *ade*6 locus. By comparing the recombination frequencies given by M26 and M375 (Table 1) it is obvious that the increases observed in the crosses with M26 depend on the map distance of the second heteroallele involved in the crosses. In the crosses with the adjacent mutants M216, L555, and L415, M26 yields only 3 to 4 times more prototrophic recombinants than M375. With growing map distance to both sides of M26 (up to L406 and L463, respectively), the increase of the M26 values above the M375 values also grows larger. L406 lies at the left end of the *ade*6 recombination map and L463 is located approximately in the middle of the map. In the crosses with mutants from the right-hand part of the map (L616 to L469), no further significant increase of the M26 values above the M375 values (beyond that observed for alleles in the middle of the gene) could be observed. With some broad fluctuation, the recombination frequencies with M26 are about 13 times higher than those

[2] In Table 1 the mutants are arranged on the basis of increasing recombination frequencies with M26 and M375, respectively. In this way some insignificant differences result in their order compared to Figure 1.

with M375. The crosses with L616 and L702 are exceptions; they show a higher and a lower difference, respectively.

With the most distant markers M26 gives about 7,000 to 9,000 prototrophs per 10^6 spores. In some of these experiments spores were also plated on YEA (approximately 100 spores per plate). In accordance with the above recombination values, about 0.8% of the resulting colonies did not form the red pigment and proved to be prototrophic for adenine upon subsequent replication on MMA. In the cross M26 × L469, about 9,200 prototrophs per 10^6 were obtained. This means that 3.7% of the tetrads from this cross should have a prototrophic spore.

As a control we did the selfings M26(h^+) × M26(h^-) and M375(h^+) × M375(h^-). The results are included in Table 1. In the crosses M26(h^+) × M26(h^-) one colony was obtained on MMA out of 1.2×10^8 plated spores. No prototrophs were found in the crosses M375(h^+) × M375(h^-).

In addition, we tested M26 for spontaneous reversibility by plating 2.2×10^8 vegetative cells on MMA (about 8×10^6 cells per plate). No reversions were found. However, reversions can be induced in M26 with $NaNO_2$ (for experimental techniques, see GUTZ 1963, p. 75). We obtained 25 reversions to prototrophy out of 6.2×10^8 cells surviving $NaNO_2$ treatment (the inactivation in the experiments was about 80%). Twelve of these reversions proved to be due to suppressors which are either unlinked or not closely linked with the *ade*6 locus. It was also found (GUTZ, unpubl. results) that M26 can be suppressed by class 1 and class 2 supersuppressors isolated by BARBEN (1966). Therefore, M26 is most probably a nonsense mutation (as already mentioned, this mutant is noncomplementing).—It should be emphasized that none of the suppressors restores M26 to a growth similar to the wild type. Strains carrying M26 and a suppressor still form the red pigment on YEA (although in most cases with less intensity than M26) and show a reduced growth rate on MMA in comparison to *ADE*6 strains.

The peculiarity of M26 to give high numbers of prototrophic recombinants in intragenic crosses might not be an attribute of the mutation in the *ade*6 locus, but might be due to an additional mutation present in the original strain. As will be described in sections II to V, a total of 555 *ade*6-M26 spore cultures from crosses with M26 strains were subjected to various tests which did give more or less detailed information on their recombination behavior. All spore cultures yielded recombination frequencies similar to the original M26 mutant. This result strongly indicates that the property "high recombination frequency" is an attribute of the *ade*6 mutation present in M26 and is not due to an additional outside mutation.

II. *Tetrad analyses from crosses of* ade*6-M26 with the wild type and two nonidentical alleles:* Because of the many prototrophs given by M26 in intragenic crosses, we tested also whether or not this allele shows high frequency of gene conversion. In tetrad analyses from crosses of M26 with the wild type about 5% aberrant tetrads were found. It was therefore of interest to analyze a large number of tetrads from crosses with M26. In addition to the wild type, M26 was crossed also with the nonidentical alleles M216 and L52. As a control we analyzed tetrads from crosses of M216, L52, and M375 with the wild type.

In these experiments aberrant tetrads can be easily detected on the basis of the red pigment formed by *ade*6 colonies. It was previously found (GUTZ 1963; LEUPOLD and GUTZ 1965) that, if plated on YEA, most *ade*6 mutants develop an intensive dark red color, whereas some of the complementing mutants develop a light red (pink) color only. The latter mutants are not leaky; they are found only within the mutants mapping in the left part of the *ade*6 map. M26 and M375 form dark red colonies; M216 and L52 were chosen because of their light red colonies. Colonies of both types of mutants (e.g., dark red versus light red) can be distinguished easily from each other as well as from the white wild-type colonies.

In our previous work it was also found that the color differences are specific for the *ade*6 alleles in question; they are a stable and unambiguous property under the experimental conditions used. Additional evidence for these facts will be given in the present paper. In the crosses no markers were included which influence the pigment formation of *ade*6 mutants (e.g., genes for arginine requirement); the presence or absence of the *ura*1 marker does not influence the colony colors at all. It should be mentioned also, that in *S. pombe* spontaneous cytoplasmic petites do not occur (in *Saccharomyces* they interfere with the pigment formation of red adenine mutants; ROMAN 1956).—Due to the color differences, postmeiotic segregation can be detected in the present system through sectored colonies (white/red or light red/dark red).

In Table 2a the crosses are shown from which tetrads were analyzed. We used for each type of cross several different strains including seven strains which had been obtained from tetrads with an aberrant *ade*6 segregation (see MATERIALS AND METHODS). This was done to test whether the conversion frequency at the *ade*6 locus might vary with the strains used. All crosses except three were heterozygous for *ura*1.

In about 74% of the dissected tetrads all four spores germinated. The completely germinated tetrads were carefully checked for aberrant segregations with respect to the color of their colonies. All tetrads with an aberrant segregation were picked up for further testing. In the event of sectored colonies we isolated both components by replating on YEA. A total of 159 tetrads with an aberrant color segregation was found. We tested these tetrads for the segregation of the mating-type and *ura*1 alleles. In 17 tetrads one or both of these loci also showed an aberrant segregation. We discarded these tetrads and they are not included in Tables 2a and 2b.

The "false" tetrads might be due to some general anomalies in meiosis and spore formation or to errors in the isolation of the spores (see FOGEL, HURST and MORTIMER 1971). Approximately equal numbers of false tetrads were observed in the crosses with and without M26: 10 were found among the 3,120 completely germinated tetrads of crosses I, II, and IV, 7 were found among the 3,769 tetrads of crosses III, V, and VI. It should be mentioned that 9 of the 17 false tetrads had sectored spore colonies; among the 142 tetrads with an aberrant *ade*6 but normal mating type and *ura*1 segregation only 3 showed postmeiotic segregation.

Table 2a shows the number of tetrads obtained with an aberrant *ade*6 segre-

TABLE 2a

Frequencies of conversion tetrads in the crosses: ade6-*M26 with the wild type and the nonidentical alleles* ade6-*M216 and* ade6-*L52;* ade6-*M216,* ade6-*L52, and* ade6-*M375 with the wild type*

Type of cross	Crossed strains	Total number of tetrads analyzed	Tetrads with aberrant color segregation*		
			Number	Percent of total number	95 percent confidence limits
(I) *ADE6* × *ade6*-M26					
	M26(h^+) × SG63	479	23	4.8 %	3.2 – 7.3%
	A802b × SG63	219	19	8.7 %	3.3 –12.5%
	L975 × A801b†	200	4	2.0 %	0.6 – 5.0%
	A301b × M26(h^-)	120	6	5.0 %	2.1 –11.6%
	Total:	1,018	52	5.11%	3.8 – 6.6%
(II) *ade6*-M216 × *ade6*-M26					
	M26(h^+) × SG146	303	14	4.6 %	2.6 – 7.8%
	SG147 × M26(h^-)	274	6	2.2 %	0.8 – 4.8%
	A801a × A801b†	91	4	4.4 %	1.2 –10.9%
	A801c × A801b	169	1	0.6 %	0.02– 3.5%
	A801a × M26(h^-)†	69	2	2.9 %	0.4 –10.0%
	A814a × A814d	126	5	4.0 %	1.3 – 9.4%
	Total:	1,032	32	3.10%	2.1 – 4.8%
(III) *ADE6* × *ade6*-M216					
	M216 × SG63	778	5	0.6 %	0.2 – 1.5%
	L975 × SG146	139	0	—	0.0 – 2.7%
	SG147 × L972	560	2	0.4 %	0.04– 1.3%
	A801a × SG63	116	0	—	0.0 – 3.2%
	Total:	1,593	7	0.44%	0.2 – 0.9%
(IV) *ade6*-M26 × *ade6*-L52					
	M26(h^+) × SG140	332	13	3.9 %	2.2 – 6.8%
	A802b × SG140	235	9	3.8 %	1.8 – 7.4%
	SG141 × M26(h^-)	300	9	3.0 %	1.4 – 5.6%
	SG141 × A801b	193	7	3.6 %	1.5 – 6.6%
	Total:	1,060	38	3.58%	2.3 – 5.4%
(V) *ADE6* × *ade6*-L52					
	L975 × SG140	374	1	0.3 %	0.01– 1.5%
	SG141 × L972	658	2	0.3 %	0.04– 1.1%
	Total:	1,032	3	0.29%	0.1 – 0.8%
(VI) *ADE6* × *ade6*-M375					
	M375(h^+) × SG63	908	8	0.9 %	0.4 – 1.7%
	A301b × M375(h^-)	229	2	0.9 %	0.1 – 3.3%
	Total:	1,137	10	0.88%	0.4 – 1.6%

* See Table 2b for the segregation in the aberrant tetrads.—As explained in the text, in crosses II and IV not all types of conversion tetrads are detectable on the basis of the color of the spore colonies, which in principle are possible.

† In these crosses both strains were *URA*1.

TABLE 2b

Segregation in the conversion tetrads of crosses I to VI

Type of cross	Number of conversion tetrads*	Number tested†	Segregation‡ Color§	Segregation‡ Genotype
(I) *ADE6* ×	46	16	3 w : 1 dr	3 + : 1 M26
ade6-M26	6	6	1 w : 3 dr	1 + : 3 M26
(II) *ade6*-M216 ×	29	17	3 lr : 1 dr	3 M216 + : 1 + M26
ade6-M26	3	3	1 lr : 3 dr	1 M216 + : 3 + M26
(III) *ADE6* ×	4	1	3 w : 1 lr	3 + : 1 M216
ade6-M216	3	1	1 w : 3 lr	1 + : 3 M216
(IV) *ade6*-M26 ×	19	19	1 dr : 3 lr	1 M26 + : 3 + L52
ade6 L52	1	1	3 dr : 1 lr	3 M26 + : 1 + L52
	1	1	3 dr : 1 lr	2 M26 + : 1 M26 L52 : 1 + L52
	13	8	1 w : 1 dr : 2 lr	1 + + : 1 M26 + : 2 + L52
	1	1	1 w : 2 dr : 1 lr	1 + + : 2 M26 + : 1 + L52
	1	1	2 w : 1 dr : 1 lr	2 + + : 1 M26 + : 1 + L52
	1	1	1 w/dr : 1 dr : 2 lr	1 + +/M26 + : 1 M26 + : 2 + L52
	1	1	1 dr/lr : 1 dr : 2 lr	1 M26 +/+ L52 : 1 M26 + : 2 + L52
(V) *ADE6* ×	2	1	3 w : 1 lr	3 + : 1 L52
ade6-L52	1	1	1 w : 3 lr	1 + : 3 L52
(VI) *ADE6* ×	3	1	3 w : 1 dr	3 + : 1 M375
ade6-M375	6	1	1 w : 3 dr	1 + : 3 M375
	1	1	1 w : 1 w/dr : 2 dr	1 + : 1 +/M375 : 2 M375

* See Table 2a for total number of tetrads analyzed.—With respect to crosses II and IV, see footnote(*) of Table 2a.

† Number of tetrads in which the shown genotypes were verified by appropriate crosses (see text). If the genotypes of the spores could be deduced from the colony colors, in general only part of the tetrads were tested.

‡ In cases of postmeiotic segregation (sectored spore colonies), two phenotypes and genotypes are shown separated by a slant.

§ Colors of spore colonies: w, white; lr, light red (pink); dr, dark red.

gation (mating type and *ura*1 segregation normal) in the various crosses. Table 2b shows the segregations in detail.—As can be seen from Table 2a, the mutation *ade*6-M26 yields, in crosses with the wild type as well as in crosses with non-identical alleles, about 3 to 5% aberrant tetrads. In each type of cross no significant differences were found among the conversion frequencies of single crosses done with different strains (a possible exception might be A801c × A801b). In cross I slightly more aberrant tetrads seem to have occurred (5.1%) than in crosses II and IV (3.1% and 3.6%, respectively), but the differences are not statistically significant. In contrast to *ade*6-M26, the mutations *ade*6-M216, *ade*6-L52, and *ade*6-M375 yield only about 0.3 to 0.9% aberrant tetrads when crossed with the wild type. At the M26 site, conversion from mutant to wild type occurred more frequently than in the opposite direction (Table 2b).

To ensure the genotypes as shown in Table 2b, some tests were necessary with the aberrant tetrads. *ADE6* spore colonies could have been simulated by mutations in adenine genes which affect steps prior to *ade6* in purine biosynthesis. Such mutants do not form the red pigment regardless of whether or not they are *ade6*. Therefore, we tested the tetrads of crosses I, III, V, and VI and also those with white spore colonies of cross IV for adenine requirement. All white spore colonies were prototrophic for adenine (the red ones required adenine for growth). Thus, none of these aberrant tetrads were caused by mutations in "early" adenine genes.

After this potential source of error had been excluded, the genotypes of most aberrant tetrads could be deduced from the color segregation. This is true for crosses I, III, V, and VI in which single site mutants were crossed with the wild type, and for the 3 lr : 1 dr and 1 w : 2 lr : 1 dr[3] tetrads of crosses II and IV, respectively. In the remaining aberrant tetrads the color segregation did not allow definitive conclusions on the genotypes (e.g., in tetrads being 1 lr : 3 dr one of the dark red spore colonies could have been a two site mutation). We tested these tetrads by appropriate crosses (see next paragraph). As a control, part of the tetrads with conclusive color segregations were also subjected to allele and suppressor tests. The number of tetrads checked is indicated in Table 2b.

We examined the tetrads in the following way. Cultures from red spore colonies were allele tested with the *ade6* allele or alleles used in the respective cross. In one 1 lr : 3 dr tetrad of cross IV a two site mutation was found. All other red segregants tested were single site mutations; in all instances the results of the allele tests confirmed the color segregation (*ade6*-M26 colonies were dark red; *ade6*-M216 and *ade6*-L52 colonies were light red). White spore colonies were checked for the possible presence of suppressors by crossing them with a wild strain; in none of the colonies tested was a suppressor present.—Further experiments with part of the aberrant tetrads are described below (Section III).

It has to be pointed out that in the two factor crosses II and IV not all aberrant tetrads, which in principle are possible, could be detected on the basis of the spore colony colors. Colonies of *ade6*-M26-L52 (and presumably *ade6*-M216-M26) have a dark red color and cannot be distinguished from M26 colonies. Therefore, tetrads which might have had two light red colonies and, instead of an M26 colony, a colony with a two-site mutation would not have been detected. In order to see whether or not such tetrads occur frequently, we allele tested from cross IV a series of 213 consecutive tetrads[4] with completely germinated spores. Of these tetrads, 208 showed a segregation 2 lr : 2 dr spore colonies, and 5 a segregation 3 lr : 1 dr. The tetrads were tested for their adenine requirement (all spore colonies required adenine for growth), uracil requirement, and mating type. In 212 of the tetrads *ura1* and mating type segregated 2 : 2; one tetrad was 3 h^+ : 1 h^- (the *ade6* and *ura1* segregations were normal; this tetrad is not included in Tables 2a and 2b).

The spore colonies of the 213 tetrads were allele tested with *ade6*-M26 and

[3] Meaning of symbols: w, white; lr, light red (pink); dr, dark red.

[4] 101 tetrads were of the strain combination A802b × SG140, and 112 from the combination SG141 × M26(h^-).

*ade*6-L52. All light red spore colonies turned out to be homoallelic with L52 and heteroallelic with M26, whereas all dark red spore colonies were homoallelic with M26 and heteroallelic with L52. Thus no spore colony with a two site mutation was found in this sample. Each of the 421 spore colonies being homoallelic with M26 gave approximately as many prototrophs with L52 as does the original M26 mutant. (The L52 spore colonies yielded also high numbers of prototrophs with the M26 h^+ and h^- test strains because this is an inherent property of these strains.)

In the experiments described above the allele tests always coincided with the color of the mutants on YEA; e.g., *ade*6-M216 and *ade*6-L52 strains were light red, *ade*6-M26 strains were dark red, and wild-type strains were white. Thus these tests give additional support to our previous experience that, under the experimental condition used, the differences in colony color are stable and reliable properties of the respective alleles.

III. *Fidelity of gene conversion:* In experiments with *Saccharomyces cerevisiae*, FOGEL and MORTIMER (1970) have found that the informational transfer in gene conversion occurred with complete fidelity. In the present experiments, the allele *ade*6-M26 is characterized by giving high recombination frequencies in intragenic crosses; each of the *ade*6 mutants M216 and L52 is characterized by a specific pattern of intragenic complementation. It was of interest to test in tetrads with 3 M26, 3 M216, or 3 L52 alleles, whether all three spore colonies behave identically with respect to the above properties. The tetrads used for these experiments had been first allele tested as described in the previous section.

Ten tetrads had been obtained with 3 *ade*6-M26 colonies (Table 2b). We crossed the 30 M26 strains from these tetrads with L52 and M210, and the frequencies of *ADE*6 recombinants were determined by plating free ascospores on MMA + uracil and MMA + uracil + adenine. All M26 strains yielded high numbers of prototrophs in both crosses, about $3{,}300 \times 10^{-6}$ with L52 and about $6{,}100 \times 10^{-6}$ with M210. The recombination frequencies are in good agreement with the values obtained with the original mutant M26 (Table 1).

From 17 tetrads with 3 *ade*6-M216 colonies and 15 tetrads with 3 *ade*6-L52 colonies, all M216 and L52 strains, respectively, were crossed with sets of non-identical complementing alleles. The latter were chosen in a way that they gave with M216 and L52 characteristic patterns of none, weak, and strong allelic complementation. All M216 segregants from the conversion tetrads tested gave an identical pattern of complementation; the same was true for the L52 segregants.

S. pombe crosses always contain a few diploid spores, and complementation tests are performed by streaking spores on MMA. This procedure allows simultaneous testing for complementation and intragenic recombination (for experimental details, see GUTZ 1963). In the absence of strong complementation the resulting numbers of colonies allow rough estimates of the recombination frequencies. In the above experiments no indications were obtained that any of the M216 or L52 segregants yielded high recombination frequencies similar to M26. In addition to these observations, we performed 24 quantitative determinations

of recombination frequencies. From each of the crosses II and IV, four conversion tetrads were randomly chosen. The three light red colonies of these tetrads were crossed with two nonidentical and noncomplementing alleles. All *ade*6-M216 or *ade*6-L52 segregants gave approximately the same numbers of prototrophs in parallel crosses, no enhanced values as with *ade*6-M26 were obtained. The tetrads tested quantitatively included A801, A802, and A814; spore cultures of these aberrant tetrads had been used in the crosses for tetrad analysis (Table 2a).

In summary, the experiments did not disclose any differences among alleles originating by gene conversion and their corresponding parents. In particular we did not obtain convertants to *ade*6-M26, *ade*6-M216, or *ade*6-L52 which gave altered recombination values in intragenic crosses.

IV. *Analyses of crosses of* ade*6-M26-L52 with* ADE*6 and* ade*6-M216:* In cross IV one tetrad with a two-site mutation *ade*6-M26-L52 was obtained (Table 2b). The strain has the collection No. A505a, is uracil-requiring (*ura*1), and has the mating type h^-. Colonies of A505a are dark red and therefore are indistinguishable from *ade*6-M26 colonies (as pointed out earlier, *ade*6-L52 colonies are light red). This two-site mutant is noncomplementing. A505a was crossed with the wild strain L975 (h^+) and the *ade*6 mutant M216 (h^+) to determine whether or not the two-site mutation resembles *ade*6-M26 with respect to gene conversion. From both crosses tetrads were isolated and analyzed as described earlier. Among the completely germinated tetrads, 2.7% showed aberrant color segregation (Table 3).

TABLE 3

Conversion tetrads in crosses of ade*6-M26-L52 with the wild type and* ade*6-M26*

Type of cross*	Total number of tetrads analyzed	Tetrads with aberrant color segregation†			
		Number	Percent of total number‡	Color§	Genotype‖
(VII) *ADE6* × *ade*6-M26-L52					
	199	4	2.01% (0.6–5.0%)	2 tetrads: 3 w : 1 dr 2 tetrads: 2 w : 1 lr : 1dr	2 tetrads: 3 + + : 1 M26 L52 2 tetrads: 2 + + : 1 + L52 : 1 M26 L52
(VIII) *ade*6-M216 × *ade*6-M26-L52					
	719	21	2.92% (1.8–4.5%)	3 lr : 1 dr	13 tetrads: 3 M216 + + : 1 + M26 L52 8 tetrads: 2 M216 + + : 1 M216 + L52 : 1 + M26 L52

* Crossed strains: (VII) L975 × A505a; (VIII) M216 (h^+) × A505a.

† As explained in the text, not all types of conversion tetrads are detectable on the basis of the color of the spore colonies, which in principle are possible.

‡ In parentheses: 95% confidence limits.

§ See footnote (§) of Table 2b.

‖ See text for the allele tests performed with the conversion tetrads.

In cross VII we found two tetrads 3 w : 1 dr, and two 2 w : 1 lr : 1 dr among 199 tetrads. The white spore colonies of the aberrant tetrads were adenine independent. The two dark red spore colonies were crossed with L52; they did not give prototrophs with this allele. Thus, the tetrads had the genotypes as shown in Table 3.—In cross VII tetrads would not have been detected on the basis of the colony colors which might have had, in addition to the two *ADE*6, an M26 allele instead of the two-site mutation. We tested a sample of 98 apparently normal tetrads by crossing their dark red spore colonies with L52. None of the 196 spore colonies yielded prototrophs with L52.

In cross VIII 21 tetrads out of 719 were obtained which were 3 lr : 1 dr.[5] The *ade*6 constitution of all colonies from the aberrant tetrads was determined by crossing them with M216 and L52. Two types of tetrads were present (Table 3). Colonies which were *ade*6-M216-L52 showed a light red color indistinguishable from M216 or L52 colonies. As a control we allele-tested the spore colonies of 14 of the aberrant tetrads also with M26. As expected, all dark red segregants did not yield prototrophs with M26, whereas all light red did.—The two-site convertants *ade*6-M216-L52 give allelic complementation with appropriate *ade*6 alleles.

As in the two-factor crosses II, IV, an dVII, not all types of aberrant tetrads, which in principle are possible, can be detected in cross VIII on the basis of spore colony colors. Thus the twenty-one 3 lr : 1 dr tetrads might not be all conversion tetrads which have occurred in this cross.

In cross VII only a relatively small number of tetrads have been analyzed, and a comparison of the frequency of aberrant tetrads with those in the crosses with *ade*6-M26 (Table 2a) is scarcely meaningful. In cross VIII nearly as many conversion tetrads were obtained as in cross II (Table 2a).

In addition to the tetrad analyses, a few crosses of A505a (*ade*6-M26-L52) with some other strains were analyzed by plating free ascospores. In a cross with the wild type (plating on YEA), 12 light red colonies (L52) were found out of a total of 4,594 colonies. That corresponds to 1.04% tetrads with one *ade*6-L52 spore; the value is in agreement with the two tetrads found out of 199 (Table 3). With mutants from the right-hand end of the intragenic map (e.g. M210), A505a yields approximately 1,700 prototrophs per 10^6 spores. That is about 15 times more than L52 is expected to yield in corresponding crosses, on the basis of its position in the *ade*6 map. (The corresponding control crosses could not be performed because L52 gives strong complementation with the mutants used.)

V. *Analysis of tetrads with one* ADE*6 spore from a cross* ade*6-M26* × ade*6-M210:* In cross IV, 14 tetrads with an *ADE*6 spore were obtained; in 13 of these tetrads M26 was the minority parent. In order to get further information about the minority-majority relationship in intragenic crosses with *ade*6-M26, this allele was crossed with *ade*6-M210. From this cross we *selected* asci with a prototrophic spore with the help of the technique developed by ANGEHRN (1964) and TREICHLER (1964). Young asci were placed on MMA layers. We incubated the

[5] In addition to these tetrads, two tetrads with an aberrant color segregation (one of them with postmeiotic segregation) were found which showed also an aberrant mating type and/or *ura*1 segregation. They were not included in Table 3.

layers for 16–20 hr at 30°C. The layers were then checked for asci with germinated spores. We found 58 asci with one germinated spore. The cells and non-germinated spores of these asci were separated by micromanipulation, and the MMA layers were then transferred to YEA plates. In 49 asci all 3 spores which had not germinated on MMA formed colonies on YEA. For the remaining 9 asci only 2 spores yielded colonies.

As expected, the colonies from the spores which had germinated on MMA were white on YEA and proved to be *ADE*6. The 165 colonies from the spores which had not germinated on MMA were dark red and adenine-requiring. (Colonies of M26 and M210 cannot be distinguished on the basis of their color.) All segregants were tested for their mating types. In each tetrad the mating-type alleles showed a 2:2 segregation (or a 2:1 segregation if the spore germination was incomplete). The adenine-requiring spore cultures were allele-tested with M26 and M210 to determine their genotypes.

The results are shown in Table 4. In all 49 tetrads with complete spore germination the intragenic recombination was nonreciprocal (that is no spore was found with a two-site mutation), and in about 96% of the tetrads *ade*6-M26 was the minority parent. The information given by the 9 tetrads with only 3 spores germinated is incomplete, but their segregation patterns are consistent with the above result.

VI. *Exclusion of diploid or disomic spores:* Gene conversion tetrads can be simulated by the presence of diploid or disomic spores. To conclude sections II to V, it is appropriate to mention that obviously none of the conversion tetrads listed in Tables 2 to 4 had such spores. No special tests were necessary in this respect, because the experimental techniques used reveal such spore cultures automatically, for example, by the presence of iodine positive colonies with azygotic asci. For details about these peculiarities of *S. pombe* see the publications of LEUPOLD cited in MATERIALS AND METHODS.

DISCUSSION

The results obtained with *ade*6-M26 can be summarized as follows. This

TABLE 4

Cross IX: ade*6-M26* × ade*6-M210**. *Analysis of 58 tetrads with one* ADE*6 spore*

Number of germinated spores	Number of tetrads	Segregation of the *ade*6 alleles
4	49	47 tetrads: 1 + + : 1 M26 + : 2 + M210 2 tetrads: 1 + + : 2 M26 + : 1 + M210
3	9	4 tetrads: 1 + + : 2 + M210 5 tetrads: 1 + + : 1 M26 + : 1 + M210

* Crossed strains: M26($h+$) × M210($h-$).

mutant yields, in crosses with nonidentical alleles, much higher numbers of prototrophic spores than are normally obtained among *ade*6 heteroalleles (Table 1). The mutation also yields relatively high frequencies of gene conversion: in crosses of *ade*6-M26 with the wild type, as well as with nonidentical alleles, a significantly greater number of aberrant tetrads was found than in crosses of the *ade*6 heteroalleles M216, M375, and L52 with the wild type (Table 2a). M216 and particularly M375 map close to M26. The conversion behavior of M26 is, therefore, not an attribute of its position in the *ade*6 locus, but appears to be a specific property of the mutation itself. The conversion frequencies at the M26 site were similar in the single factor cross I and in the two- and three-factor crosses II, IV, and VIII, respectively. Thus an enhanced genetic heterogeneity had no significant influence on the events leading to gene conversion.

The most striking feature of *ade*6-M26 is that its mutation site converts predominantly to the wild-type site: in all crosses taken together, conversion tetrads being 3 + : 1 M26 for the M26 site were about 12 times more frequent than tetrads being 1 + : 3 M26. In the intragenic two- and three-factor crosses, symmetrical double and triple conversions occurred frequently on all heterozygous sites (Tables 2b and 3): the site of M216 converted always together with the M26 site, whereas the more distant site of L52 showed about 60% co-conversion with M26. The presence of M26 dominates the conversion patterns of M216 and L52; in the case of L52, this is true regardless of whether this allele is in repulsion (cross IV) or coupling (crosses VII and VIII) with M26. The influence of M26 on M216 and L52 can be described by saying M26 is able to "pull" nonidentical sites into gene conversion.

In asci with a prototrophic spore (crosses IV and IX), a high degree of polarity was found: *ade*6-M26 was the minority parent in 95% of these asci; no reciprocal intragenic recombination was observed. For comparison, the data of ANGEHRN (1964) must be mentioned. He used "normal" (as in contrast to M26) mutants and found that the intragenic recombination in the *ade*6 locus was in most cases nonreciprocal, although some asci with reciprocal recombination were found. In those asci with nonreciprocal recombination, the left-hand mutant was the minority parent in approximately 77% of the cases. Thus our results show a higher degree of polarity than those of ANGEHRN. The direction of polarity is the same in both experimental sets. However, this statement is restricted to the crosses *ade*6-M26 × *ade*-6-L52 and *ade*6-M26 ×*ade*6-M210. Both L52 and M210 map to the right of M26. If the working hypothesis explained below (preferential single DNA strand breaks at the M26 site) is correct, the polarity should be reversed with respect to sites being located to the left of M26.

Taken together, all results show that *ade*6-M26 displays a pronounced site-specific marker effect with respect to intragenic recombination and gene conversion. Marker effects have been found in prokaryotic as well as eukaryotic organisms (NORKIN 1970; WHITEHOUSE 1970). Our data display aspects which are in agreement as well as in disagreement with some other results on gene conversion. Symmetrical double or multi-site conversions were found, for instance, by FOGEL, HURST and MORTIMER (1971) in Saccharomyces. These

authors consider it to be "most unlikely that these double site conversions represent independent though simultaneous events", and infer "that conversion involves a sizable segment of DNA rather than a narrowly restricted point." This explanation also appears to be valid for the symmetrical two- and three-site conversions reported in the present paper.

In the experiments of FOGEL *et al.* (1971), 3 : 1 and 1 : 3 conversion tetrads occurred with equal frequency, and differences in the conversion rates among different alleles of one gene appeared to "be correlated only with the position of the alleles in the locus and not with any other properties of the alleles." These findings are different from the present results and from data obtained with eight-spored Ascomycetes (for examples, see ROSSIGNOL 1969). FOGEL *et al.* (1971) call in question reported instances of disparity between 3 : 1 and 1 : 3 segregations because these data are based either on spore color or on colony color segregations which they consider as "potentially variable developmental attributes." However, with respect to the present data the criticism of FOGEL *et al.* is not justified. The accumulation of the red pigment in *ade*6 colonies is a stable property caused by a defined biochemical block in the purine pathway. In our numerous tests no exceptions were found with regard to colony color and *ADE*6 genotype. Furthermore, other potential sources of error (diploidy, disomy, suppressors) have been likewise excluded.

In most recombination models it is assumed that the events leading to gene conversion consist of two different steps: (1) the formation of hybrid DNA, starting at points of preferential breakage, and (2) the correction of mismatched bases (EMERSON 1969; WHITEHOUSE 1970). If a mutant shows a pronounced marker effect as *ade*6-M26, it might have an influence either on the breakage mechanism or on the correction process. ROSSIGNOL (1969), for instance, interpreted the marker effects which he observed in *Ascobolus immersus* as being caused by an influence of the mutations on the correction process; he assumed that the formation of hybrid DNA always started at a fixed breakage point outside of gene 75. With respect to the *ade*6 gene of *S. pombe*, a point of preferential breakage appears to be located outside and to the left of this gene. This may be inferred from ANGEHRN's (1964) results mentioned above.

The behavior of *ade*6-M26 can be best explained by assuming that this mutant has an influence on the breakage of DNA rather than on the correction of mismatched bases. I suppose that the DNA at the M26 site preferentially undergoes single strand breakage followed by exonucleolytic degradation towards *both* ends of the locus. It is then assumed that one free end (or both free ends) of the broken and partially degraded strand triggers events in the homologous chromatid which finally result in a conversion tetrad. As shown in Figure 2, such events can be visualized on the basis of the gene conversion model of PASZEWSKI (1970) (this model resembles in some aspects that proposed by TAYLOR 1967). The extraordinary feature of M26 appears to be that, if this allele is present in a cross, an additional point of preferential breakage is initiated within the *ade*6 locus. Thus the original mutation has not only blocked the translation of the *ade*6 gene (nonsense codon), but also seems to have changed the DNA in a way that this

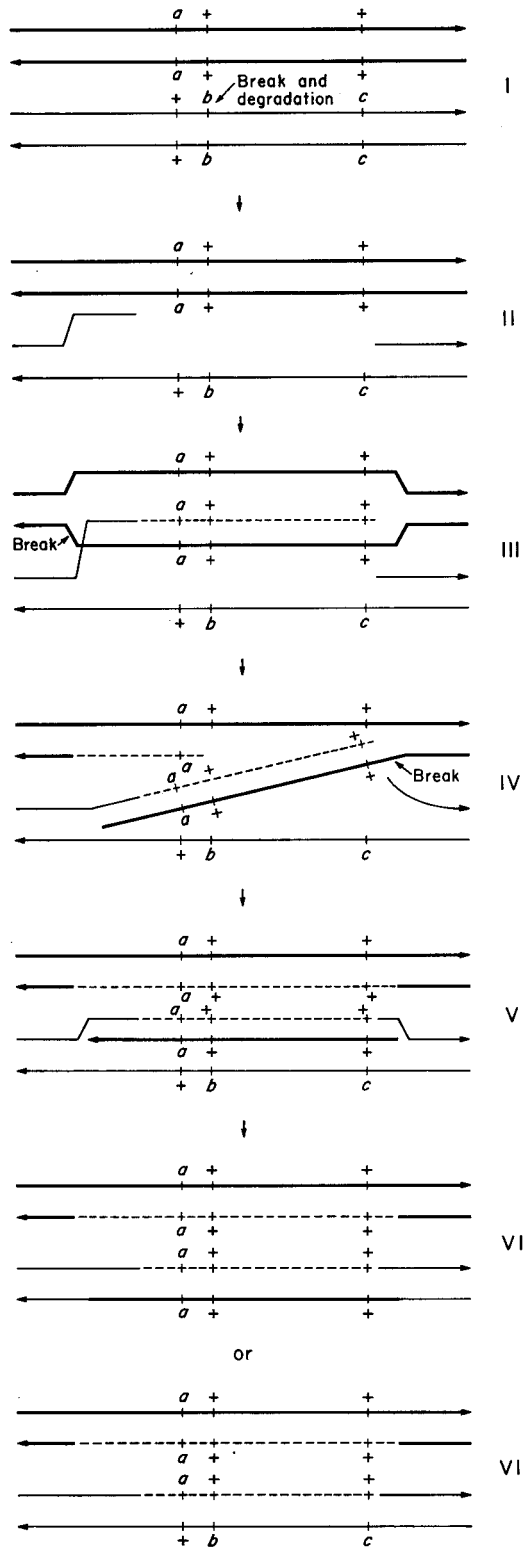
region of the gene now behaves differently with respect to the initiation of gene conversion.

On the basis of this working hypothesis, all data obtained with *ade*6-M26 can easily be explained. Because the preferential breaks would occur only in the chromatids carrying the M26 site, but not in chromatids with the wild-type site, an excess of 3 + : 1 M26 over 1 + : 3 M26 tetrads is expected (the latter tetrads might be due to conversion events which are initiated at the "normal" breakage point outside the *ade*6 locus). The influence of M26 on the conversion frequencies of sites being located to its left (M216) as well as to its right (L52) and the symmetry of the conversion are understandable if the degradation is extended at both sides of the initial break (Figure 2). The degradation appears to be extensive; the probability of the second site being included in the erosion is a function of its map distance from M26. As has been already mentioned, the working hypothesis implies an interesting prediction with respect to the polarity in asci with a prototrophic spore. If intragenic recombination in the *ade*6 locus is initiated by a single strand break at the M26 site, this allele will always be the minority parent, regardless of the position of the second allele. Whether or not this prediction is true for alleles mapping to the left of M26 remains to be determined in further experiments.

The influence of *ade*6-M26 on intragenic recombination (plating of free spores) also can be explained by assuming preferential single strand breaks at the M26 site. As the results of the tetrad analyses, the data of Table 1 also reveal an effect of M26 towards both ends of the gene. A puzzling aspect of these data is the progressive increase (across one half the gene length) of the recombination frequencies with M26 over those with M375. I did not succeed in devising a quantitative interpretation of this effect.

One aspect of our results has to be still discussed: the virtual absence of postmeiotic segregation (p.m.s.) in the crosses with *ade*6-M26. In filamentous Ascomycetes, the frequency with which p.m.s. occurs appears to be species or strain specific as well as mutant specific (WHITEHOUSE 1970; PASZEWSKI 1970). In our system p.m.s. is, at least in principle, detectable by sectored colonies. The nearly complete absence of this event could have a trivial reason: when hybrid spores germinate, one of the daughter cells might die. However, it seems improbable that any significant number of hybrid spores remained undetected because of this possibility.

HOLLIDAY (1964, 1968) and WHITEHOUSE (1965) as well as PASZEWSKI (1970) postulate in their gene conversion models that heteroduplex DNA is formed. In all three models, the absence of p.m.s. can be interpreted as an indication for the existence of a very efficient repair mechanism. However, in PASZEWSKI's model, which was used to illustrate the idea of preferential single strand breakage at the M26 site, an additional interpretation is possible. In this model (Figure 2) the length of hybrid DNA formed depends upon the extent of single strand degradation and upon the mode in which configuration V is resolved. A virtual absence of p.m.s. would result if configuration V is nearly always transformed to configuration VI.


a
b
c
Break and degradation
I
II
Break
III
IV
V
VI
or
VII

Although our results can be nicely explained by assuming a new point of preferential breakage at the M26 site, I would like to mention some thoughts which can be set forth by assuming that *ade*6-M26 does not influence the frequency with which gene conversion is initiated in the *ade*6 locus, but rather affects the repair of mismatched bases. The conversion model of HOLLIDAY (1964, 1968) will be used as basis for this part of the discussion.

As has been discussed in detail elsewhere (GUTZ 1971), an interpretation of cross I with the help of HOLLIDAY's model leads to the conclusion that in at least 8.4% of the meioses (in 85 out of 1018) the site of M26 must have been included in hybrid DNA. In 33 of these meioses (fraction $f_{min} = 0.387$) one hybrid site was corrected to wild type and the other to mutant: tetrads with a 2 : 2 segregation resulted which are not distinguishable from "true" normal tetrads. If *ade*6-M26 does not influence the frequency with which hybrid DNA is formed and if statistical variations are disregarded, the M375 site (cross VI) should have been also included in hybrid DNA in at least 8.4% of the meioses, that is, in at least 96 meioses out of 1137. Only 10 aberrant tetrads were observed in cross VI. Therefore in 86 meioses (fraction $f = 0.896$) the repair should have been in a manner that 2 : 2 asci resulted. A comparison of M26 and M216 leads to a similar result (M216 site: $f \sim 0.9$).

From the above arguments it would follow that "normal" alleles, if included in hybrid DNA, are nearly always corrected in a way that 2 : 2 asci are formed, whereas the peculiarity of *ade*6-M26 would be that this allele is preferentially repaired in a way that 3 + : 1 − tetrads result. I consider this possible explanation of our data as less likely than the postulate that M26 influences the frequency with which gene conversion is initiated in the *ade*6 locus. The preferred idea of a breakage point at the M26 site is, of course, still hypothetical and has to be substantiated in further experiments.

I thank Mrs. LOIS WILSON and Miss SHIRLEY WALKER for excellent technical assistance, and Dr. S. L. GOLDMAN for critical reading of the manuscript.

SUMMARY

The *ade*6 mutant M26 of *Schizosaccharomyces pombe* shows the following

FIGURE 2.—Illustration of the idea of preferential single strand breaks at the M26 site with the help of PASZEWSKI's (1970) gene conversion model.

The three-factor cross VIII (Table 3) is shown, the sites of *ade*6-M216, *ade*6-M26, and *ade*6-L52 are symbolized by *a*, *b*, and *c*, respectively. Only the two chromatids participating in gene conversion are drawn, each chromatid consisting of one DNA double helix. Conversion is initiated by a single strand break at the M26 site (*b*) (I), followed by degradation of the broken strand towards both ends of the gene. During chromosome pairing, the free end of this strand causes partial separation of the DNA strands in the homologous chromatid (II, III). The events proceed through stages IV and V to either stage VI or VII (dashed lines: newly synthesized DNA strands). The virtual absence of postmeiotic segregation in the crosses with *ade*6-M26 indicates that configuration V is nearly always transformed to configuration VI.

In the above example, symmetrical conversion has occurred on all three heterozygous sites resulting in a conversion tetrad 3 *a*+++ : 1 +*bc*. If *c* is not included in the degradation of the initially broken strand, a tetrad 2 *a*+++ : 1 *a*+*c* : 1 +*bc* will result.

peculiarities with respect to intragenic recombination and gene conversion. (1) If *ade*6-M26 is crossed with nonidentical alleles, up to 20 times more prototrophic spores are obtained than are expected from the recombination map distances. (2) The intragenic recombination is nonreciprocal; in asci from two-factor crosses with 1 prototrophic spore, *ade*6-M26 was the minority parent in 95% of the cases. (3) Crosses of *ade*6-M26 with the wild type as well as with nonidentical alleles yield 3 to 5% conversion asci; this amount is significantly higher than the conversion frequencies found in crosses of other *ade*6 alleles with the wild type. (4) In intragenic two- and three-factor crosses which include the site of M26, symmetrical double and triple conversions occur frequently. The presence of M26 "pulls" nonidentical sites into conversion events. (5) In the conversion asci from the various crosses with *ade*6-M26, a great disparity between 3 : 1 and 1 : 3 tetrads was observed. The M26 site converted predominantly from mutant to wild type. (6) The conversion behavior of *ade*6-M26 is caused by some specific properties of this allele and does not depend on any recombination genes which might have been present in the original M26 mutant (e.g., genes which are not allelic with *ade*6 but which influence gene conversion in this locus). (7) The allele *ade*6-M26 is suppressible and therefore is most probably a nonsense mutation. (8) Alleles which had originated from gene conversion showed the same properties as their parent alleles.—The marker effect displayed by *ade*6-M26 can be best explained by assuming that single DNA strand breaks occur preferentially at the M26 site.

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