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The Strong *ADH1* Promoter Stimulates Mitotic and Meiotic Recombination at the *ADE6* Gene of *Schizosaccharomyces pombe*

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The effect of the strong promoter from the alcohol dehydrogenase gene on mitotic and meiotic intragenic recombination has been studied at the *ade6* locus of the fission yeast *Schizosaccharomyces pombe*. A 700-bp fragment containing the functional *adh1* promoter was used to replace the weak wild-type promoter of the *ade6* gene. Analysis of mRNA showed that strains with this *ade6*::*adh1* fusion construct had strongly elevated *ade6*-specific mRNA levels during vegetative growth as well as in meiosis. These increased levels of mRNA correlated with a 20- to 25-fold stimulation of intragenic recombination in meiosis and a 7-fold increased prototroph formation during vegetative growth. Analysis of flanking marker configurations of prototrophic recombinants indicated that simple conversions as well as conversions associated with crossing over were stimulated in meiosis. The strongest stimulation of recombination was observed when the *adh1* promoter was homozygous. Studies with heterologous promoter configurations revealed that the highly transcribed allele was the preferred acceptor of genetic information. The effect of the recombinational hot spot mutation *ade6-M26* was also investigated in this system. Its effect was only partly additive to the elevated recombination rate generated by the *ade6*::*adh1* fusion construct.

The mechanisms of genetic recombination have been investigated in great detail in the last few years. Although much information has been collected and several models for recombination have been proposed (e.g., references 26, 27, 31, 32, and 40), the mechanisms are not yet fully understood. However, it has become clear that several aspects of DNA metabolism, such as DNA repair and transcription, are closely interrelated with recombination.

Transcription and recombination studies have been done particularly in eucaryotes. The results show that recombination between segments of the immunoglobulin heavy-chain locus is accompanied by transcription. A role in targeting of the recombination events has been proposed for this activity (3, 46, 47). In addition, recombination between adenovirus DNA and hamster, mouse, or human cell DNA is also found to occur at transcriptionally active regions. It was proposed that transcription establishes a chromatin configuration which is more accessible to foreign DNA and for the recombination machinery (35).

In the budding yeast *Saccharomyces cerevisiae*, several systems that show a correlation between transcription and recombination have been reported. Mating-type switching occurs via a double-strand break which is induced by the HO endonuclease only in the expressed cassette of the mating-type locus (38). However, if the two remaining mating-type cassettes that are normally transcriptionally silent become expressed as the result of a mutation in one of the four *SIR* genes (18, 21), they too serve as recipients for the HO-induced double-strand cut (20). Recently it has been shown that the *SIR2* gene not only is involved in the control of expression of the mating-type system but also represses both mitotic and meiotic intrachromosomal recombination in the ribosomal DNA repeats (10).

From this ribosomal DNA cluster, a sequence (*HOT1*) has been isolated which stimulates genetic exchange of DNA when inserted at locations outside of the ribosomal DNA repeats (19). *HOT1* consists of two elements, a polymerase I initiation site and an enhancer of polymerase I transcription (8, 9, 44). Both elements are required for transcription as well as for the stimulation of recombination, suggesting a strong correlation between these two processes (37).

A different approach to the study of recombination dependent on transcription was used by Thomas and Rothstein (41). They measured recombination between direct repeats of *GAL10* sequences in *S. cerevisiae*. Cells that were expressing these genes exhibited a 15-fold increased loss of sequences between the repeats. However, conversion was not affected in this system.

So far, all investigations concerning the connection between transcription and recombination have been done in mitosis. The effect of promoter sequences and transcription on meiotic recombination is not yet known. To investigate this question, we replaced the weak *ade6* promoter of *Schizosaccharomyces pombe* by the strong promoter from the *adh1* gene (33, 34) and analyzed meiotic intragenic recombination at *ade6* dependent on the different transcription activities. mRNA levels from vegetatively growing cells as well as from meiotic cells were correlated with recombination frequencies.

In addition to normal *ade6* mutations, the recombinational hot spot mutation *ade6-M26* was studied in this system. This mutation per se stimulates intragenic recombination 10-fold in meiosis and shows a strong disparity in favor of conversion from mutant to wild type (12), but it has no effect during vegetative growth of the cells. The *adh1* promoter was fused with this *ade6-M26* mutant allele, and the construct was used in crosses to test the joint influence of the strong promoter and the recombinational hot spot activity.

MATERIALS AND METHODS

Strains and plasmids. All yeast strains used are from the strain collection in Bern or were constructed in this study. All strains have the *ura4* gene deleted and are therefore *ura4-D18* (11). The DNA sequence of the *ade6* gene has been

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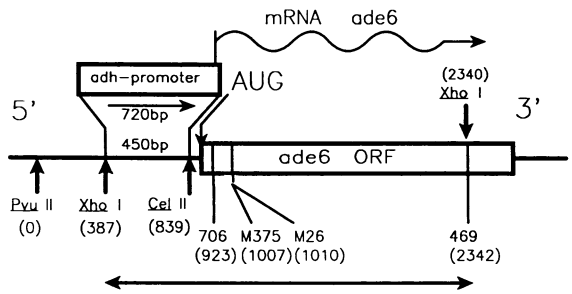


FIG. 1. Replacement of the *ade6* promoter by the *adh1* promoter. Numbers in parentheses give positions of the restriction sites or *ade6* mutations with respect to the published sequence (39). The 1.95-kb fragment between the *Xho*I sites (indicated by a double-sided arrow) was used to probe the RNA used for Fig. 2 and 3.

determined (39). Position 875 represents the A of the initiation codon ATG (39). The mutations *ade6-M375* (position 1007), *ade6-M26* (position 1010), and *ade6-469* (position 2342) were shown to be point mutations by DNA sequence analysis (29, 39). *ade6-706* has been cloned but not sequenced (39). Since this mutation destroys an *Hae*III site and is suppressible by nonsense suppressors, it is most likely a C-to-T transition at position 923 and therefore also a point mutation. The *ade6* 5′-flanking marker *ura4-aim* (artificially introduced marker) is the *ura4* gene artificially introduced 15 kb proximal to *ade6* (10a). *tps16* (14) was used as a marker on the 3′ side of *ade6*. Plasmid pUC8 (43) was used for all in vitro constructions. The constructs were renamed pCG, followed by an isolation number. Plasmid pURA3-1.1 (15) was used as the selecting plasmid in cotransformation experiments. Plasmids were amplified by using *Escherichia coli* JA221 (2).

Standard biochemical and genetic methods. A description of standard genetic methods and media for *S. pombe* is given by Gutz et al. (13). As a selection medium for adenine prototrophs, we used YEA supplemented with 200 mg of guanine per liter (6, 30). DNA manipulation was done by standard procedures (24). The lithium acetate protocol of Ito et al. (17) was used to transform *S. pombe*.

Construction of *S. pombe* strains that have replaced the *ade6* promoter by the *adh1* promoter. A 720-bp *Sph*I-*Eco*RI fragment containing the active *adh1* promoter as well as the start site for mRNA synthesis (cloned by Russell and Hall [34]) was ligated into the *Xho*I (position 387)-*Cel*II (position 839) sites of wild-type *ade6* DNA. This led to the exchange of the *ade6* promoter for the *adh1* promoter (Fig. 1). The mRNA made by the natural promoter starts at positions 815 to 830 (39), producing an untranslated region of 46 to 60 nucleotides. The *adh1* mRNA start site lies 66 bp upstream of the *adh1* ATG initiation codon and 8 bp within the fragment used for the replacement (33). Therefore, transcription of the *ade6* gene by the *adh1* promoter should lead to an untranslated region of about 50 bases, comparable with the normal situation at the *ade6* locus.

An appropriate DNA fragment containing the *adh1* promoter and flanking *ade6* sequences was used to transform *S. pombe* GC1. In this strain, the *ura4*$^+$ gene has replaced a 2.2-kb *Eco*RV-*Bam*HI fragment on the 5′ side of *ade6* (10a). It is phenotypically Ura$^+$ and Ade$^-$. *S. pombe* transformants were obtained by selecting for adenine prototrophy. Southern analysis confirmed the correct replacement of the inserted *ura4* gene by the transformed *adh1* promoter fragment, yielding strain *ade6*$^+$::*adh1* (data not shown). Strain *ade6-469*::*adh1* was constructed in the same way, using *ade6-469*::*ura4* as the recipient. These transformants were obtained on 5-fluoro-orotic acid plates, on which uracil auxotrophs can be selected (11).

The mutations *ade6-706*, *ade6-M375*, and *ade6-M26* are close to the 5′ end of the gene (39). Therefore, the *adh1* promoter had to be integrated in a different way in these mutant alleles. All three mutant alleles were cloned into plasmid vectors and recombined in vitro with the *adh1* promoter, using appropriate restriction enzymes. The recombinant *S. pombe* DNA was then isolated from the plasmids and used to cotransform strain *ade6*$^+$::*adh1 ura4-D18* together with pURA3-1.1 (15). After selection for uracil independence, the transformants (usually 4,000 to 10,000) were replicated onto YEA, a full medium containing a limited amount of adenine. Because Ade6$^-$ mutants grow red on this medium, transformants that have incorporated the *ade6* mutant allele during cotransformation are easily detected by their formation of a red pigment. Such colonies were picked, reisolated, and analyzed by restriction digestion and hybridization of genomic DNA (data not shown).

Construction of diploid strains. Two sets of four diploids were constructed. Set 1 diploids were homozygous for the temperature-sensitive *pat1-114* mutation, which allows the synchronization of meiosis in fission yeast cells (16, 28). These strains were used to synchronize meiosis and to prepare RNA from meiotic cells. Set 2 diploids are wild type with respect to *pat1* and were used to determine the mitotic recombination frequencies shown in Table 5. As a control, we also measured the meiotic recombination frequencies of these strains (see Table 5). The *ade6* genotypes of the diploids (see Table 5) are identical for both sets. Set 1 diploids were constructed by using *ura4-aim* and *leu1* as complementation markers. *ura4-aim* and *tps16* were used to construct set 2 diploids. The two sets were constructed by the same procedure but with different selective media and temperatures (because of the different markers used). Two appropriate haploid strains that carried different *ade6* mutations and complementation markers were plated together on fresh crossing medium. After the formation of the first zygotes (at 6 to 10 h, according to microscopic observation), the cells were removed and plated on a minimal medium containing adenine and incubated at 22°C (set 1 diploids, containing the temperature-sensitive mutation *pat1-114*) or at 35°C (set 2 diploids). Because of intergenic complementation, only cells that had previously formed zygotes by fusion of the two differently marked haploids on the crossing medium could grow under these conditions. Diploidy of these cells was checked under the microscope and on Phloxin B (0.002%; Sigma)-containing medium (13). To obtain stable h^-/h^- diploids (for measurement of the mitotic recombination frequencies), the cells were grown in liquid medium. A mitotic crossover between the centromere of chromosome II and the *mat* locus led to stable h^+/h^+ or h^-/h^- diploids. Such cells were screened by plating the cells on complete medium, replicating the colonies to sporulation medium, and checking their ability to produce spores. Colonies that did not produce spores were reisolated, tested again for diploidy and mating type, and stored at 4°C.

Determination of mitotic recombination rates. Five colonies from each set 2 diploid were picked and suspended in water. Appropriate dilutions were plated on selective and nonselective plates to assess the fraction of prototrophic cells in each colony. The median method of Lea and Coulson (22) was used to determine mitotic recombination rates.

Meiotic recombination frequencies. Meiotic intragenic re-

combination in crosses of haploids was measured as described previously (13). For determination of the meiotic frequencies in diploid strains, h^+/h^- diploids were plated onto sporulation medium and incubated at 25°C for 3 days. The mixture of vegetative cells and azygotic asci was treated as in crosses with haploid cells, and the spores were plated onto selective and nonselective media.

Gene conversion and the association of crossover. Haploid strains were crossed, and prototrophic recombinants were selected. They were picked and tested on appropriate media and temperatures for their flanking marker configurations. Prototrophs with the parental flanking markers originally introduced with the 5′ *ade6* mutation were designated 5′ convertants, whereas prototrophs with the parental markers entering with the 3′ *ade6* mutation were termed 3′ conversions. Prototrophs that had exchanged their flanking markers were considered convertants with an associated crossover.

Analysis of mRNA from vegetatively growing cells. Total RNA was prepared from the following haploid strains: (i) *ura4-D18 h⁻*, (ii) *ade6⁺::adh1 ura4-D18 leu1-32 h⁻*, (iii) *ade6-M26 ura4-D18 h⁻*, (iv) *ade6-M26::adh1 ura4-D18 leu1-32 h⁻*, (v) *ade6-M375 ura4-D18 h⁻*, and (vi) *ade6-M375::adh1 ura4-D18 leu1-32 h⁻*. The strains were grown in minimal medium supplemented with uracil and leucine (each at 100 mg/liter) and adenine (10 mg/liter) to a density of 5×10^6 to 8×10^6 cells per ml, cooled on ice, and separated into aliquots of 50 ml. The tubes were kept on ice between all manipulations. Cells were washed once with water and resuspended in 1 ml of LETS 1% buffer (10 mM Tris hydrochloride [pH 7.4], 10 mM dilithium EDTA, 1% [wt/vol] lithium dodecyl sulfate). An equal volume of glass beads (0.5-mm diameter) was added, and the tubes were vigorously vortexed six times for 20 s each time. After addition of 4 ml of LETS 0.2% buffer (LETS 1% but with 0.2% lithium dodecyl sulfate), the solution was immediately extracted with an equal volume of PCIA (phenol-$CHCl_3$-isoamyl alcohol [25:24:1]–0.1% [wt/vol] 8-hydroxychinoline, saturated with 0.1 M Tris hydrochloride [pH 7]–0.2% [wt/vol] 2-mercaptoethanol). The phases were separated by centrifugation, and the extraction was repeated until no interphase was visible (two or three times). After addition of LiCl to a final concentration of 0.1 M, nucleic acids were precipitated with 2.5 volumes of ethanol. The step was repeated twice by addition of LiCl to a final concentration of 0.2 M and ethanol to selectively precipitate the RNA. The yield of RNA in each preparation was determined by measuring A_{260}. RNA was stored in water at −70°C. A 1-ml sample of culture yielded 5 to 8 g of RNA.

Total RNA was analyzed essentially as described by Maniatis et al. (24), applying the glyoxal-dimethyl sulfoxide denaturation procedure with the following modifications. A 10-g sample of total RNA was denaturated in a mixture of 6% (vol/vol) glyoxal, 50% (vol/vol) dimethyl sulfoxide, and 35 mM NaH_2PO_4 (pH 6.5) by incubation for 15 min at 50°C. After the RNA solution was cooled on ice, 1/5 volume of loading buffer (50% glycerol, 25 mM NaH_2PO_4, 0.4% bromphenol blue) was added. A 1.2% agarose gel poured in 25 mM NaH_2PO_4 buffer was used for separation and run with 4 V/cm for 4 h. The RNA transfer on nitrocellulose filter was carried out immediately after electrophoresis and without further treatment of the gel. As a hybridization probe, we used 1.5×10^6 cpm of the nick-translated 1.95-kb *Xho*I *ade6* fragment (Fig. 1).

Preparation of RNA from meiotic cells. To generate cultures in mid-exponential growth phase, fresh patches of the set 1 diploids (homozygous for *pat1-114*) were used to inoculate 10 ml of liquid minimal medium supplemented with 10 mg of adenine per liter. After incubation at 24°C for 48 h, 100 ml of the same medium was inoculated with 1 ml of the preculture. These cultures were grown at 24°C to a density of 2×10^6 to 4×10^6 cells per ml (about 24 h). The temperature was then rapidly shifted to 34°C by shaking the cultures under a jet of hot water. Incubation was continued in a 34°C water bath shaker. Four hours after the temperature shift, total RNA from 60 ml of these cultures was prepared as described above. A 1-ml sample of initial culture yielded 2 to 4 g of total RNA. The residual 40 ml of each culture was further incubated at 34°C, and the synchronicity of meiosis was checked 8 and 10 h after the temperature shift by microscopically determining the ratio between asci and vegetative cells. Total RNA of cultures with more than 90% of ascus-producing cells 8 h after the temperature shift and more than 90% complete asci after 10 h was used for Northern (RNA) blotting as described above. Thus, RNA was analyzed only from well-synchronized cultures in which more than 90% of the cells had entered meiosis after the temperature shift.

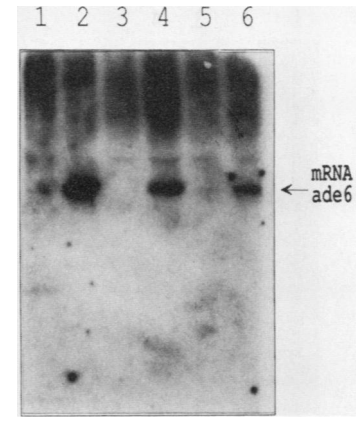


FIG. 2. Northern analysis of RNA from vegetative cells. Each lane contained 10 g of total RNA Lanes: 1, *ade6⁺* wild type; 2, *ade6⁺::adh1*; 3, *ade6-M26*; 4, *ade6-M26::adh1*; 5, *ade6-M375*; 6, *ade6-M375::adh1*.

Rehybridization of Northern blots. The nitrocellulose filters used for Fig. 2 and 3 were washed for 4 h at 80°C in 0.1% sodium dodecyl sulfate. Rehybridization was done with 1.5×10^6 cpm of the nick-translated plasmid pSp4C/2, which contains the *pho1* gene (25).

RESULTS

Mitotic and meiotic cells contain high levels of *ade6*-specific mRNA when the *adh1* promoter is fused to the *ade6* gene. To determine the mRNA levels in vegetatively growing cells, the haploid strains (as indicated in the legend to Fig. 2) were grown to the exponential phase, and total RNA was subsequently prepared as described in Materials and Methods. A 10-μg sample of total RNA was loaded in each lane of Fig. 2 (amounts of RNA were determined by measuring A_{260}). The results indicated that wild-type cells contained only small amounts of *ade6* mRNA during vegetative growth. However, in the strains containing the *ade6::adh1* fusion construct, very high levels of *ade6* mRNA were detected, suggesting that the *ade6* gene is heavily transcribed in these strains (see Discussion).

To examine the amount of *ade6* mRNA in meiosis, we

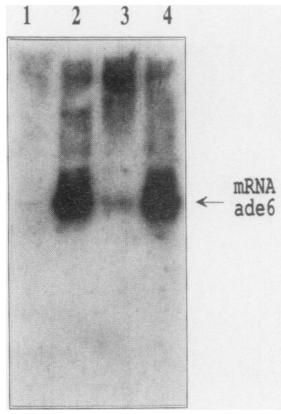


FIG. 3. Northern analysis of RNA from meiotic cells (diploids). Each lane contained 8 g of total RNA. Lanes: 1, *ade6-469/ade6-706*; 2, *ade6-469::adh1/ade6-706::adh1*; 3, *ade6-469/ade6-M26*; 4, *ade6-469::adh1/ade6-M26::adh1*.

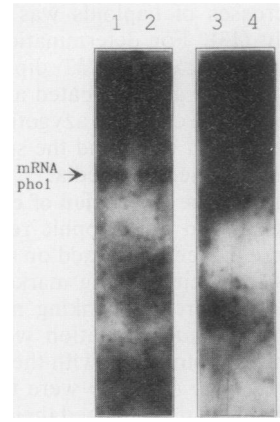


FIG. 4. Control hybridization. The filters used for Fig. 2 and 3 were rehybridized with the radioactively labeled *pho1* gene (see Materials and Methods). Lanes: 1, ade$^+$ wild type (corresponds to lane 1 of Fig. 2; RNA from vegetative cells); 2, *ade6$^+$::adh1* (corresponds to lane 2 of Fig. 2; RNA from vegetative cells); 3, *ade6-469/ade6-706*, diploid strain (corresponds to lane 1 of Fig. 3; RNA from meiotic cells); 4, *ade6-469::adh1/ade6-706::adh1*, diploid strain (corresponds to lane 2 of Fig. 3; RNA from meiotic cells).

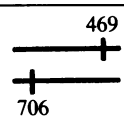
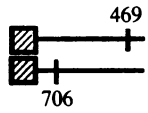
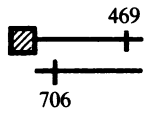
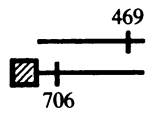
used the set 1 diploids (see Materials and Methods). These diploids are homozygous for the temperature-sensitive mutation *pat1-114*. At the restrictive temperature, the protein kinase encoded by *pat1* is inactivated, resulting in activation of the *mei2* gene, which in turn triggers premeiotic DNA synthesis and meiosis (7). A shift of diploid cells homozygous for *pat1-114* from the permissive to the restrictive temperature allows the synchronized entry of *S. pombe* cells into meiosis, since under these circumstances the start of the meiotic cycle is independent of the growth conditions. Total RNA was prepared 4 h after the shift to the restrictive temperature, that is, about 1 h before the first meiotic division takes place (34a). RNA was used for Northern analysis only from cultures showing more than 90% ascus-producing cells 8 h after the temperature shift and more than 90% complete asci after 10 h of incubation at the restrictive temperature (see Materials and Methods). Therefore, the Northern blot shown in Fig. 3 contains RNA prepared mostly (>90%) from meiotic cells, and contamination with mitotic cells that had failed to enter meiosis was at most weak. Since the same amount of RNA (8 μg) was loaded in each lane of Fig. 3, the strong signals detected in lanes 2 and 4 suggests that strains containing the *ade6::adh1* fusion construct had high levels of *ade6* mRNA also in meiosis (see Discussion).

As a control for the *ade6* message on the Northern blots, we washed the filters used for Fig. 2 and 3 and rehybridized them with the radioactively labeled *pho1* (25) gene (Fig. 4). We analyzed the rehybridized RNA from lane 1 (*ade6$^+$* wild type) and lane 2 (*ade6$^+$::adh1*) of Fig. 2 that derived from vegetatively growing haploid cells as well as rehybridized RNA from lane 1 (*ade6-469/ade6-706*) and lane 2 (*ade6-469::adh1/ade6-706::adh1*) of Fig. 3. These RNA preparations originated from diploid cells of synchronized meiotic cultures (see Materials and Methods). The following conclusions can be drawn from the hybridization pattern with *pho1* (Fig. 4). (i) Vegetatively growing cells contained a small amount of *pho1*-specific mRNA (Fig. 4, lanes 1 and 2); (ii) no mRNA of the *pho1* gene was detectable with our methods in RNA prepared from cells of synchronized meiotic cultures (lanes 3 and 4); (iii) the *pho1* mRNA levels were not affected by the fusion of the *adh1* promoter to the *ade6* gene; and (iv) at least in lanes 1 and 2 (since we detected no signal in lanes 3 and 4, this conclusion cannot be drawn for these RNAs), the same amount of RNA was loaded for the Northern blot shown in Fig. 2. This latter point shows directly that the strong signals, specific for *ade6* mRNA that was detected in lane 2 of Fig. 2, could not have been due to a higher amount of loaded RNA.

Increased intragenic recombination between *ade6::adh1* mutant alleles. The crosses made to assess the influence of the strong *adh1* promoter on recombination are shown in Tables 1 to 4. In crosses involving normal alleles, i.e., alleles without the *M26* marker effect (Tables 1 and 2), intragenic recombination was strongly stimulated by the *adh1* promoter. The strongest stimulation (factor 25) was obtained when the promoter was homozygously present in the cross, independent of the positions of the crossed *ade6* mutations. With heterozygous promoters, the stimulation dropped (except for cross 3 in Table 1) to approximately the square root of the value observed in the homozygous case. When *ade6-M26* (which increases intragenic recombination frequencies up to 20-fold) was used, further stimulation by the *adh1* promoter was at most weak (Tables 3 and 4).

The highly transcribed allele is the acceptor of genetic information. To determine the direction of gene conversion, we used the two flanking markers, *ura4-aim* and *tps16* (10a). A parental flanking marker configuration of a prototrophic recombinant was taken to indicate conversion from mutant to wild type of the *ade6* allele originally associated with the respective outside markers. Tables 1 and 2 show the results of crosses with the mutations *ade6-706*, *ade6-M375*, and *ade6-469*, which do not show the increased meiotic recombination activity of the hot spot mutation *ade6-M26*. The results with the latter mutation are given in Tables 3 and 4 (see below). The control crosses (crosses 1 in Tables 1 and 2) exhibited a polarity of gene conversion; i.e., one of the mutations in each cross was more frequently converted to wild type than was the other. If the *adh1* promoter was present in both alleles, the direction of the polarity remained qualitatively the same, but in the cross with widely separated alleles it was even more pronounced (Table 1). However, the site in *cis* to the *adh1* promoter was always preferentially converted if it was heterozygous in the cross. This led to a

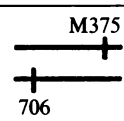
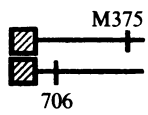
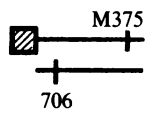
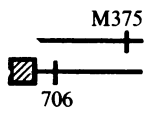
TABLE 1. Effect of the *adh1* promoter on intragenic recombination between *ade6-706* and *ade6-469*

Cross	Alleles involved[a]	No. of Ade$^+$ recombinants/ 10^6 spores	Factor of stimulation	Sample size[b]	Parental flanking marker arrangements of Ade$^+$ recombinants[c]		
					Frequency (%)	No. of conversions/10^6 spores	
						706→+	469→+
1		400		191	28	94 (5)	18 (1)
2		10,000	25	188	34	3,200 (20)	160 (1)
3		360	1	189	44	69 (1)	89 (1.3)
4		1,800	4.5	189	51	790 (6)	130 (1)

[a] Hatched boxes indicate the presence of the *adh1* promoter. Alleles without boxes are under the control of the *ade6* promoter.
[b] Number of Ade$^+$ recombinant clones tested with respect to outside markers. Sample is assumed to be random.
[c] The alleles involved in conversion were deduced from the flanking marker combinations described in Materials and Methods. The relative frequencies of the two conversion classes (given in parentheses) were determined by setting the number of occurrences in the minority class at 1.

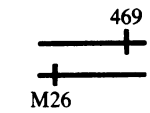
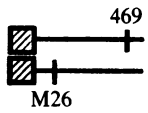
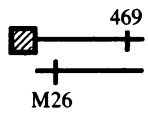
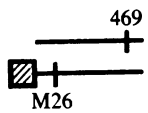
reversal of the normal polarity in these crosses (crosses 3 and 4 versus cross 1 in Tables 1 and 2). As can be calculated from Tables 1 and 2, the alleles in *trans* to the *adh1* promoter were converted with about the same frequencies as in the control crosses with the exception of *ade6-469* in cross 4 of Table 1: although this allele was under the control of the *ade6* promoter, its conversion to wild type was enhanced sevenfold. A similar, although not as significant, stimulation of the conversion frequency of an allele in *trans* to the *adh1* promoter was seen in cross 4 of Table 2: *ade6-M375* was converted twice as efficiently as in the control. This *trans*-stimulating effect was detected for the promoter-distal alleles

TABLE 2. Effect of the *adh1* promoter on intragenic recombination between *ade6-706* and *ade6-M375*[a]

Cross	Alleles involved	No. of Ade$^+$ recombinants/ 10^6 spores	Factor of stimulation	Sample size	Parental flanking marker arrangements of Ade$^+$ recombinants		
					Frequency (%)	No. of conversions/10^6 spores	
						706→+	M375→+
1		17		91	43	3.4 (1)	4.1 (1.2)
2		390	23	192	51	90 (1)	110 (1.2)
3		77	4.5	192	58	2.7 (1)	42 (15)
4		58	3.4	161	57	25 (3)	8 (1)

[a] For explanations, see footnotes to Table 1.

TABLE 3. Effect of the *adh1* promoter on intragenic recombination between *ade6-M26* and *ade6-469*[a]

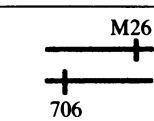
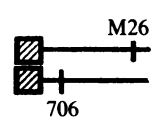
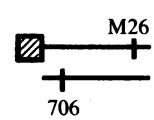
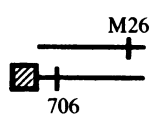
Cross	Alleles involved	No. of Ade$^+$ recombinants/ 10^6 spores	Factor of stimulation	Sample size	Parental flanking marker arrangements of Ade$^+$ recombinants		
					Frequency (%)	No. of conversions/10^6 spores	
						M26→+	469→+
1		7,700		191	40	2,600 (5.5)	470 (1)
2		16,000	2	191	34	5,200 (21)	250 (1)
3		4,600	0.6	190	47	2,000 (11)	180 (1)
4		2,800	0.4	192	49	1,200 (8)	150 (1)

[a] For explanations, see footnotes to Table 1.

only. One explanation for this observation could be that asymmetric heteroduplex DNA formed in the vicinity of the promoter would have had the ability to switch to symmetric heteroduplex DNA with a certain probability. If so, a widely separated intragenic mutation could frequently have been converted by events that were initiated in *trans*. Slightly decreased conversion frequencies were observed for the 5′ mutation in *trans* to the *adh1* promoter (crosses 3 in Tables 1 and 2). Conversion of these mutations could be associated with conversion of *adh1* promoter sequences. Since the promoters were heterozygous in these crosses, conversion of only a part of them could have produced a nonfunctional hybrid promoter and thus have led to adenine dependence. Such events were not detectable with our system and would therefore have reduced the measured recombination frequencies.

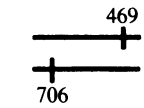
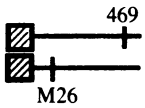
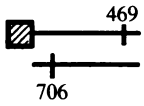
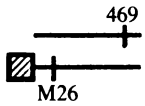
The *adh1* promoter also stimulates mitotic recombination at *ade6*. As in meiosis, vegetatively growing cells showed a

TABLE 4. Effect of the *adh1* promoter on intragenic recombination between *ade6-706* and *ade6-M26*[a]

Cross	Alleles involved	No. of Ade$^+$ recombinants/ 10^6 spores	Factor of stimulation	Sample size	Parental flanking marker arrangements of Ade$^+$ recombinants		
					Frequency (%)	No. of conversions/10^6 spores	
						706→+	M26→+
1		200		192	55	8 (1)	100 (12)
2		560	2.8	192	45	105 (1)	147 (1.4)
3		140	0.7	192	66	5 (1)	87 (17)
4		410	2	192	65	77 (1)	190 (2.5)

[a] For explanations, see footnotes to Table 1.

TABLE 5. Effect of the *adh1* promoter on mitotic and meiotic recombination in diploid strains

Diploid	Alleles involved[a]	No. of mitotic prototrophs formed/cell/ generation (10^6)	Factor of stimulation	No. of meiotic prototrophs/ spore (10^6)	Factor of stimulation
1		11		600	
2		10	1	5,500	9
3		76	7	8,600	14
4		ND[b]	ND	10,500	2[c]

[a] See Table 1, footnote *a*.
[b] ND, Not determined.
[c] Stimulation with respect to diploid 2.

strongly elevated *ade6*-specific mRNA level when this gene was controlled by the *adh1* promoter (Fig. 2). To investigate whether this observation correlates with an increased rate of mitotic recombination, we constructed the set 2 diploids (Materials and Methods). The same genetic flanking markers as in the crosses with haploid strains (Tables 1 to 4) were involved. The median method of Lea and Coulson (22) was used to determine the recombination rates of these strains. A sevenfold increased rate was obtained with the diploid strain that had the *adh1* promoter fused to both *ade6* copies (Table 5). As a control, we allowed the same diploids to sporulate on crossing medium and measured the intragenic recombination frequencies in meiosis. The mean values of two independent sets of experiments are given in Table 5. The factor of meiotic stimulation was 14 (Table 5, diploid 3 versus diploid 1). This lower enhancement than in the crosses with haploid strains (Table 1) may have been due to the diploid nature of the strains.

The stimulating effects of the *adh1* promoter and *M26* are not additive. The recombinational hot spot mutation *ade6-M26* increases *ade6* intragenic recombination 10-fold in meiosis (12). We fused the *adh1* promoter to this mutation to investigate whether the *M26* marker effect is influenced by this strong promoter. We observed a stimulation of meiotic recombination in crosses of *M26* with *ade6-469* (Table 3) or *ade6-706* (Table 4) when the *adh1* promoter was fused to both alleles. But this stimulation was only weak (factor of 2) compared with the values obtained in crosses not involving *M26* (Tables 1 and 2). When the promoter was heterozygous, recombination frequencies apparently were even reduced.

In the crosses without *M26* (Tables 1 and 2), the mutation in *cis* to the *adh1* promoter was always more frequently converted. However, a different situation was observed in crosses involving *M26*. The *M26*-containing allele always was the preferred acceptor of wild-type information, independent of its relation (*cis* versus *trans*) to the *adh1* promoter (Tables 3 and 4).

To test whether the absence of the *M26* effect in mitosis (29) was dependent on the promoter, we constructed the diploids shown in Table 5 and measured their mitotic recombination rates. As expected, the mitotic rate of prototroph formation was not elevated in the *M26* diploid with the *ade6* promoters (Table 5, diploid 2 versus diploid 1). Unfortunately, no mitotic frequencies could be obtained from diploid 4 (homozygous *adh1*; *M26/469*). This strain was leaky and could therefore grow on selective medium. The same phenomenon has been reported by Ponticelli (28a), working with a multicopy plasmid carrying *ade6-M26* in an *ade6-469* strain.

DISCUSSION

Several publications reporting an influence of transcription on recombination have appeared (35–37, 41, 44). The results of these experiments indicate that transcriptionally active DNA is responsible for an elevated recombination rate in mitosis. To our knowledge, no work has been done to assess whether there is a correlation between transcription and recombination in meiosis.

In this work, we replaced the weak *ade6* promoter of *S. pombe* by the strong promoter from the *adh1* gene cloned by Russell and Hall (34). Since promoters were exchanged in different *ade6* alleles, we were able to determine intragenic recombination in mitosis and meiosis in dependence of the different promoters.

High levels of *ade6* mRNA in strains with the *ade6*::*adh1* gene construct. We found by Northern analysis that the *ade6*::*adh1* gene construct mediated high levels of *ade6* mRNA in mitosis as well as meiosis (Fig. 2 and 3). Although we were not measuring transcription rates, we believe for several reasons that the *ade6* gene was indeed heavily transcribed by the *ade6*::*adh1* construct. The high levels of *ade6* mRNA detected in these cells could, in principle, also be due to an accumulation of stable mRNA products.

However, since we prepared mitotic RNA from exponentially growing cells, in the mRNA for steady-state conditions, synthesis and decay of the message should be more or less balanced (Fig. 2). The strong signals in lanes 2, 4, and 6 of Fig. 2 could therefore be interpreted to indicate that the *ade6* gene did indeed have an increased transcription rate in the strains with the fusion constructs. That the same amount of RNA was loaded in each lane is shown by the internal control with *pho1* (Fig. 4, lanes 1 and 2).

For the meiotic RNA (Fig. 3), however, we had no steady-state conditions. Three explanations are possible for the increased intensity of the meiotic signals in the *ade6*::*adh1* strains. (i) Mitotic cells that had failed to enter meiosis could have contaminated the meiotic culture. The signals would be partly mitotic and partly meiotic. (ii) Preexisting message, produced in mitosis, may not have been decayed in the time between the temperature shift (to initiate meiosis) and the RNA preparation. The signals would be partly mitotic also in this case. (iii) The *ade6*::*adh1* fusion genes could have been heavily transcribed in meiosis. The signals would reflect mostly meiotic RNA only in this case. Since for the Northern blots we used the prepared RNA only from cultures with more than 90% asci (examined 10 h after the temperature shift; see Materials and Methods), contamination of the meiotic cultures with mitotic cells was at most weak. In addition, the control hybridization with *pho1* (Fig. 4) indicates that differences between mitotic and meiotic RNA preparations are detectable with our system (no *pho1* message in the meiotic preparation; Fig. 4, lanes 3 and 4). Mitotic *pho1* mRNA did not survive the 4 h at 34°C between the temperature shift and the RNA preparation. Thus, with the caveat that mitotically synthesized *ade6* mRNA might not decay in meiosis, as does *pho1* mRNA, we conclude that the *ade6* mRNA present in meiotic cells (Fig. 3) are transcripts synthesized during meiosis.

The *ade6*::*adh1* gene construct mediates enhanced intragenic recombination at *ade6* in vegetatively growing and meiotic cells. We determined intragenic recombination frequencies between two widely separated *ade6* mutations in meiosis (Table 5) and mitosis (Table 2) in the context of different promoters. In addition, the meiotic frequencies were measured between two closely spaced mutations (Table 2) and in crosses involving the recombinational hot spot mutation *ade6-M26* (Tables 3 and 4; see below). In crosses without this hot spot mutation, meiotic recombination was stimulated 25-fold when the *adh1* promoter was homozygous but to a much lesser extent in heterozygous conditions (Tables 1 and 2). Two reasons could account for the observation that the sum of the recombination frequencies of the heterozygous crosses was smaller than the frequency with the homozygous *adh1* insertions. The large sequence dissimilarity in the promoter region could disturb recombination by preventing a correct pairing of homologs or by blocking the formation and extension of conversion tracts. The second reason could be that transcription is needed in both strands to generate recombinogenic DNA structures. In our crosses without the recombinational hot spot mutation *ade6-M26* (Tables 1 and 2), we found that the highly transcribed strand was the acceptor for information. This finding could mean that a more open DNA or chromatin structure mediated by RNA polymerase II transcription is more easily invaded by a donor DNA strand. If this donor strand is also heavily transcribed (as in the crosses homozygous for the *adh1* insertion), it should have the same open DNA structure, allowing an enhanced interaction with its homolog in meiosis.

In their mitotic study with duplicated *GAL10* genes that were separated by plasmid sequences, Thomas and Rothstein (41) observed an enhanced frequency of plasmid loss when the genes were transcribed. However, conversion of information from one copy to the other seemed not to be affected. Here we show that meiotic conversions with and without crossover association were stimulated equally in the *ade6* gene by our fusion construct with the strong *adh1* promoter. This follows from the fact that the relative frequencies of parental and recombinant flanking marker arrangements among prototrophic recombinants were at most slightly changed when the *ade6* promoter was replaced by the *adh1* promoter.

However, if the promoter was heterozygous, the association of crossovers was reduced (in the range of 10%; Tables 1 to 4, crosses 1 and 2 versus crosses 3 and 4). The reason for this is not clear. The 700-bp heterology in the promoter region could prevent the extension of conversion tracts. There is some evidence that shorter conversion tracts are less frequently associated with crossovers (1). The heterology would therefore not have reduced the frequency of associated crossovers per se but rather would have reduced the formation of large conversion tracts, which in turn were more often resolved by a crossover.

The stimulation of the recombination frequencies in vegetatively growing cells was less pronounced but still sevenfold (Table 5, diploid 3 versus diploid 1). A direct influence of active transcription on recombination in mitotic cells has been proposed for *S. cerevisiae* (37, 41). Here we show a correlation between elevated mitotic recombination frequencies and high levels of mRNA also in fission yeast. Nevertheless, we cannot exclude the possibility that not the *adh1*-specific transcription activity but some other factors, such as special sequence motifs possibly inserted with the promoter fragment, could account for the stimulation of the recombination frequencies. However, at least for the meiotic situation, there is weak evidence that the promoter sequence per se was not responsible for the increase in the recombination frequencies but that its presence (or probably its activity) might have stimulated the normal recombination activity at *ade6*. If the newly introduced sequence contained a strong initiation site for recombination, one would expect a mutation close to this site to exhibit higher conversion frequencies than does a widely separated mutation. Control cross 1 (Table 2) demonstrated that the *M375* mutation converted 1.2 times more frequently to wild type than does the *706* mutation, although its distance to the promoter is about 100 bp greater than that of *706*. This weak 1:1.2 polarity remained the same even if the promoters of both *ade6* genes were exchanged by *adh1* promoters, which were responsible for a more than 20-fold-increased recombination activity. Although very little is known about the phenomenon of polarity, our observation could be interpreted to indicate that the normal recombination activity remains qualitatively the same, independent of the present promoter. The *adh1* promoter might therefore stimulate recombination by increasing the normal recombination activity at *ade6* (probably by enhanced transcription of the gene; see above) rather than by bringing in a new and strong initiation site for gene conversion. However, additional two-point crosses with other mutant alleles in the 5′ region of the *ade6* gene should be carried out to distinguish between true polarity and simple marker effects due to differential mismatch repair.

The effects of the recombination-stimulating mutation *ade6-M26* and the enhanced recombination activity mediated by the

***adh1* promoter are not additive.** The *adh1* promoter strongly stimulates intragenic recombination at *ade6*. However, if *M26* was involved in the cross, the frequency of prototrophs was only doubled compared with the values for controls lacking the *adh1* promoter (Tables 3 and 4). Possibly even a reduction of the frequencies was obtained when the promoter was heterozygously present.

The hot spot mutation alone stimulates recombination by a factor 10 to 20 (crosses 1; Tables 3 versus 1 and 4 versus 2; 14). An additional twofold stimulation was produced by the *adh1* promoters, leading finally to a 40-fold enhanced intragenic recombination frequency when both the *M26* mutation and the *adh1* promoters were present (cross 1 of Table 1 versus cross 2 of Table 3). However, a 250-fold stimulation would be expected if the two elements acted independently of each other.

The finding of a heptameric sequence at *M26* that is absolutely required for the effect (18a) supports the hypothesis that *M26* is a binding site for a protein that influences the initiation of meiotic recombination at that site, although this has not yet been shown directly. On the other hand, the role of strong transcription in general recombination is not yet clear. One possibility is that the transcription activity of RNA polymerases may change the structure of the DNA in a way that it is more accessible for the recombination machinery (35, 37, 41). Continuous transcription would therefore be needed to retain the recombinogenic structure of the DNA. However, an *M26*-specific DNA structure needed for the observed high recombination activity could be lost by enhanced *adh1*-mediated transcription. Another possibility is that strong transcription prevents an *M26*-specific protein from binding to DNA. Whatever constitutes the effect of *M26*, it is not fully additive to the transcription-mediated stimulation of recombination.

Another possible reason for the only partial synergism of the two effects is the requirement of additional important elements in *cis* for *M26* action. The *adh1* promoter could either have replaced such sequences or altered their correct spacing with respect to *M26*. Alternatively, the observed phenomenon could be the result of a saturation effect on recombination.

At the moment, we cannot distinguish among these possibilities. Systematic mutagenesis of the *ade6* promoter region as well as of sequences further up- or downstream of the gene could provide an answer to the question of additional genetic elements in *cis* to *M26*.

The connection between transcription and recombination. Schulz et al. (35) have shown in their studies with mammalian cells that the cellular DNA sequences at junction sites of integrated viral DNA are transcriptionally active. They propose that an accessible DNA structure is generated by transcription of these sequences, which in turn would facilitate the attachment of recombination proteins. In their studies, the transcribed sequences are the recipients of genetic information.

In our crosses without the recombinational hot spot mutation *M26*, we made a similar observation. The strongly transcribed allele was preferentially the acceptor for information during gene conversion. Because of the unchanged direction of polarity of gene conversion in crosses with homozygous *adh1* promoters (crosses 1 and 2 of Tables 1 and 2), it is possible that the natural recombination activity at *ade6* is not altered but merely stimulated by the strong promoter, possibly because of a change in chromosome structure.

It has been proposed that transcription may affect the superhelical structure of the DNA (23). The isolation of plasmids that are highly supercoiled from *top1* mutants of *S. cerevisiae* (4) and the detection of supercoiled domains near transcriptionally active sequences (42, 45) support this idea. It has also been shown that the breaking and rejoining activities of topoisomerase I can be uncoupled by special substrates (reviewed in reference 5). Thus, it is possible that topoisomerases create DNA lesions that are recombinogenic when releasing torsional stress after transcription.

Alternatively, transcription of DNA by RNA polymerase II could simply make pairing of homologs and strand exchange more likely by loosening the tight structure of the DNA double helix. More experiments have to be done to establish the role of transcription in the recombinational process. Supplementing the mitotic studies in *S. cerevisiae* (37, 41), we provide evidence from our experiments in fission yeast that transcription may influence recombination also in meiosis.

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