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Genetic Evidence for Preferential Strand Transfer During Meiotic Recombination in Yeast

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ABSTRACT

During meiotic recombination in the yeast *Saccharomyces cerevisiae*, heteroduplexes are formed as an intermediate in the exchange process. In the formation of an asymmetric heteroduplex, one chromosome acts as a donor of a single DNA strand and the other acts as a recipient. We present genetic evidence that the nontranscribed strand is donated more frequently than the transcribed strand in spores that have an unrepaired mismatch at the *HIS4* locus.

IN the yeast *Saccharomyces cerevisiae*, three different types of meiotic segregation are commonly observed in tetrads derived from a diploid that is heterozygous for a genetic marker (alleles *A* and *a*): (1) 2*A*:2a (Mendelian segregation), (2) 3*A*:1*a* or 1*A*:3*a* (gene conversion) and (3) tetrads in which one or more spores shows postmeiotic segregation (PMS). Although higher frequencies are observed for some mutational changes at some loci, for an average allele, about 5% of the unselected tetrads represent conversion events and 0.25% represent PMS events (FOGEL, MORTIMER and LUSNAK 1981). PMS events reflect the segregation of the two alleles from a single haploid spore at the first mitotic division following meiosis. Spores that show PMS presumably contain chromosomes with heteroduplex DNA at the *A* locus, with one strand of *A* information and one strand of *a* information (HOLLIDAY 1964). In yeast, PMS events are usually detected by tetrad dissection of a diploid strain that is heterozygous for an auxotrophic marker. The spores are allowed to form colonies on a rich growth medium (allowing growth of both wild type and mutant cells) and then replica-plated onto medium lacking the required growth factor. Sectored colonies on this medium indicate PMS events (ESPOSITO 1971).

PMS and gene conversion events share a number of properties (FOGEL, MORTIMER and LUSNAK 1981). About half of both events are associated with reciprocal crossing-over of flanking markers; because of this association, heteroduplexes are likely to be a necessary intermediate in the process of crossing-over (HOLLIDAY 1964; MESELSON and RADDING 1975). In *S. cerevisiase*, the amount of DNA transferred between two homologous chromosomes in a conversion or PMS event has been estimated by studying how frequently two closely linked markers show co-conversion or co-PMS. Such studies (FOGEL, MORTIMER and LUSNAK 1981) indicate that the amount of DNA transferred for both conversion and PMS events is approximately 1 kb, although both larger and smaller amounts have also been observed (DICAPRIO and HASTINGS 1976; BORTS and HABER 1987; JUDD and PETES 1988; SYMINGTON and PETES 1988).

The relationship between gene conversion and PMS events is somewhat controversial. In some models of recombination (HOLLIDAY 1964; MESELSON and RADDING 1975; RADDING 1982), conversion is the result of mismatch repair in heteroduplexes (Figure 1a). Alternatively, it has been suggested that conversion events may be the result of repair of a double-stranded gap (SZOSTAK *et al.* 1983), rather than mismatch repair (Figure 1b). By the first type of model, therefore, the low level of PMS (for most alleles) relative to conversion is explained by the efficient repair of mismatches. In the second model, a low frequency of PMS relative to conversion is expected if the size of the double-stranded gap is larger than the region of heteroduplex. As described below, most evidence supports the first type of model.

One argument that indicates conversion is the result of mismatch repair in heteroduplexes is that the ratio of the frequencies of PMS and gene conversion appears to be related to the individual mismatch. WHITE, LUSNAK and FOGEL (1985) found that the *arg4-16* mutation, which shows a high level of PMS (48% of the aberrant segregations; FOGEL, MORTIMER and LUSNAK, 1981) is a G to C transversion. Depending on which DNA strand is transferred, in a diploid heterozygous for *arg4-16* and a wild type *ARG4* gene, a heteroduplex formed at the *ARG4* locus would be expected to contain either a G/G or C/C mismatch. Other *arg4* mutant alleles, resulting in other types of mismatches, had low levels of PMS. In experiments in which heteroduplexes (constructed *in vitro*) were introduced into mitotically dividing yeast cells, BISHOP,

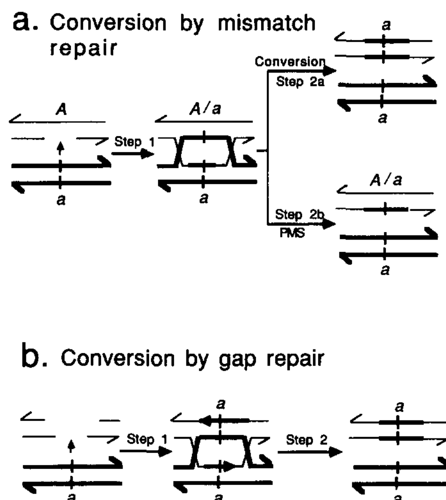

FIGURE 1.—Models of recombination. Recombination between two of the four chromatids is shown. The mutant alteration in the DNA is indicated by a short vertical line. (a) RADDING'S (1982) modification of the Meselson-Radding model. The recombination event is initiated by a nick on the upper chromosome that is expanded into a single-stranded gap. Step 1: A single-strand derived from the chromatid with the *a* allele invades the gap, resulting in a heteroduplex containing a mismatched base. The single-stranded ends from the upper chromosome invade the lower DNA molecule. One of the ends is used as a primer for repair DNA synthesis (indicated by the arrow). Step 2: The double-Holliday structure can be resolved in a variety of ways by cutting strands at the junctions. The mode of cutting determines whether the region of heteroduplex will be associated with a cross-over of flanking DNA sequences (SZOSTAK *et al.* 1985); in this figure, a cross-over is not associated with the region of heteroduplex. Step 2a: The mismatch in the heteroduplex is repaired by excision of the wild type strand and repair synthesis using the mutant strand as a template. This type of repair would result in a gene conversion event. Step 2b: The mismatch is not repaired. Segregation of this chromosome into a spore would result in a PMS event. (b) Double-strand break repair model of recombination (SZOSTAK *et al.* 1985). Recombination is initiated by a double-strand break that is expanded into a gap. Step 1: The broken ends of the upper chromosome invade the lower chromosome and repair synthesis occurs on both chromosomes (indicated by arrows). Although, in the original model, most conversion is the result of gap repair, conversion could result from mismatch repair, if the mismatch occurred in the small region of heteroduplex (flanking the double-stranded gap) resulting from strand invasion; PMS events result from unrepaired mismatches in the heteroduplex. Step 2: The structure is resolved by cleaving the single DNA strands as described for the Radding model.

ANDERSON and KOLODNER (1989) found that C/C, A/A and T/T mismatches were infrequently repaired relative to G/G mismatches. In similar experiments, B. KRAMER *et al.* (1989) found C/C mismatches are less efficiently repaired than any other mismatch. The inefficient repair of C/C mismatches is also a characteristic of mismatch repair in prokaryotic systems (reviewed by WHITE, LUSNAK and FOGEL (1985)).

Since large deletions do not show PMS (FOGEL, MORTIMER and LUSNAK 1981), it is somewhat surprising that two of the alleles with very high levels of PMS are *ade8-18* (54% PMS of total aberrant segregations), which is a 38-bp deletion (WHITE, LUSNAK and FOGEL 1985), and *his4-lop* (72% PMS) (NAG, WHITE and PETES 1989), which is an insertion of a 26 bp palindromic oligonucleotide. It has been suggested that the high PMS at *ade8-18* is the result of a binding site for a protein that prevents mismatch repair that is fortuitously formed as the result of the deletion (WHITE *et al.* 1988). If a heteroduplex is formed between a wild type gene and a gene containing an insertion or deletion, a single-stranded loop is formed. The high PMS frequency of *his4-lop* (and other palindromic insertions such as *his4-lopc*) appears to result from a "hairpin" structure formed by intrastrand pairing within the 26-bp loop (NAG, WHITE and PETES 1989). This structure is apparently poorly recognized by the mismatch repair system.

A second indication that conversion reflects mismatch repair in heteroduplexes is the phenotype of the *pms* mutants (WILLIAMSON, GAME and FOGEL 1985). In these mutants, the frequency of PMS events is increased and the frequency of gene conversion decreased at many (although not all) heterozygous loci. The deduced amino acid sequence of the *PMS1* gene indicated homology to the *mutL* protein of Salmonella and the *hexB* protein of Streptococcus, proteins known to be required for mismatch repair in these organisms (W. KRAMER *et al.* 1989).

Although the experiments described above suggest that, at least in yeast, most conversion is the result of mismatch repair of heteroduplexes (consistent with the model of recombination shown in Figure 1a), it is possible that meiotic recombination initiates by a double-strand break. The repair of the break could involve a relatively small double-strand gap but extensive single-strand excision and heteroduplex formation (SUN *et al.* 1989; THALER and STAHL 1988).

In *S. cerevisiae*, in a tetrad with a PMS event, in general, only a single spore with PMS is observed (FOGEL, MORTIMER and LUSNAK 1981). The nomenclature for PMS tetrads in yeast is borrowed from eight-spored fungi (since PMS events were first detected in such fungi); in this nomenclature, each half of a spore colony is counted as a meiotic segregant. The most common types of PMS tetrads in yeast are those that segregate $2A{:}1a{:}1A/a$ spore colonies ($5A{:}3a$ tetrad) and those that segregate $2a{:}1A{:}1A/a$ spore colonies ($3A{:}5a$ tetrad). Tetrads with two PMS events (for example, $1A{:}1a{:}2A/a$ spore colonies (aberrant 4:4 tetrad)) are usually rare in yeast. Since aberrant 4:4 tetrads are much less frequent than 5:3 or 3:5 tetrads, FOGEL, MORTIMER and LUSNAK (1981) argued that heteroduplex formation in yeast usually involves only a single chromatid (an asymmetric heteroduplex) rather than symmetric involvement of two chromatids as postulated by some models of recombination (HOLLIDAY 1964); similar observations and conclusions

were also made in Ascobolus by STADLER and TOWE (1971).

In the formation of an asymmetric heteroduplex such as that shown in Figure 1a, in principle, either the transcribed DNA strand or the nontranscribed strand could be donated to the recipient chromosome. We describe below genetic evidence that, although either strand can be donated, there is a preference for donating the nontranscribed strand in spores that have an unrepaired mismatch at the *HIS4* locus. This genetic study complements a previous investigation involving the physical analysis of heteroduplex DNA, which indicated a preference for transfer of the nontranscribed strand at the *ARG4* locus of yeast (M. LICHTEN, C. GOYON, A NICOLAS, N. P. SCHULTES, D. TRECO, J. W. SZOSTAK and J. HABER, personal communication).

MATERIALS AND METHODS

Strains: The strains used in this study were derivatives of AS4 (*MATα trp1 arg4 tyr7 ade6 ura3 MAL2*) and AS13 (*MAT***a** *leu2 ade6 ura3*). These strains were constructed (by A. STAPLETON, University of Chicago) from the haploid strains LS18 and LS25-70d (SYMINGTON and PETES 1988) by multiple transformations. In addition to auxotrophic markers, these strains contain multiple restriction site changes between *LEU2* and *CEN3*. The diploid strain DNY26 used in our study was isogenic (except for changes introduced by transformation) with a diploid produced by crossing AS4 and AS13. Further details of the construction of DNY26 are given by NAG, WHITE and PETES (1989). In brief, the haploid AS13 was transformed with a plasmid (pDN13) that contained the selectable *URA3* gene and a 26-bp palindromic insertion in the *Sal*I site of the *his4* gene. Ura^- derivatives of this transformant in which the mutant *his4* gene had replaced the wild type allele were selected (two-step transplacement; SCHERER and DAVIS 1979). The resulting haploid was mated to AS4 to generate the diploid DNY26.

Media and genetic techniques: Most of the genetic procedures used in this study were standard (SHERMAN, FINK and HICKS 1982), except for the meiotic analysis. Cells were grown vegetatively in YPD medium (1% yeast extract, 2% Bacto-peptone, 2% dextrose) overnight and were then transferred to plates containing sporulation medium (1% potassium acetate, 0.1% yeast extract, 0.05% dextrose, 2% agar) supplemented with adenine and uracil (6 μg/ml). These plates were incubated at 18° until tetrads had formed (3–5 days). Tetrads were treated with glusulase and dissected on YPD plates or SD-his plates (solid medium containing 0.17% Difco yeast nitrogen base (without ammonium sulfate or amino acids), 0.5% ammonium sulfate, 2% dextrose, 2% agar, all required amino acids and bases (concentration of about 0.1 mg/ml) except histidine).

The tetrads that were dissected onto SD-his plates were incubated at 32° for about 9 hr and were then examined microscopically. In most of the tetrads, two of the spores failed to divide or bud and the other two spores had divided three times; all spores that had budded or divided one or more times were scored as His^+. After scoring all spores, we transferred (using sterile spatulas) the SD-his medium containing the spores to a plate containing YPD overlayed with 0.4 ml of a 0.5% histidine solution. The plates were incubated at 32° for 3–4 days and then replica-plated to SD-his medium. After 1 day, all colonies on the SD-his plate were examined with the microscope in order to detect small sectors. The pattern of sectoring was then correlated with the phenotype of the spore to generate the data shown in Table 2.

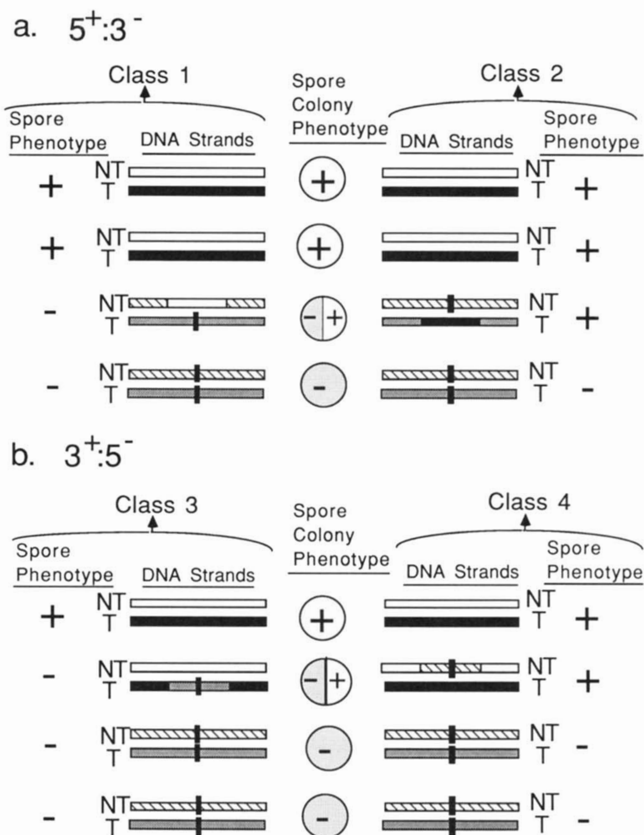

FIGURE 2.—Patterns of strand transfer in $5^+:3^-$ and $3^+:5^-$ tetrads. The interaction of two chromatids is shown; each DNA strand is shaded differently. The mutant substitution is indicated by short vertical lines. T and NT indicate the transcribed and nontranscribed strands and + and − indicate the wild type and mutant phenotypes. (a) Tetrads with a single PMS event of the $5^+:3^-$ type are most readily explained as the result of an unrepaired heteroduplex in which a single DNA strand is donated from the wild-type gene to the mutant gene. In forming the heteroduplex that will eventually give rise to a sectored colony, the donated strand could be either nontranscribed (class 1) or transcribed (class 2). If the donated strand is nontranscribed, the spore should be His^-, since wild type gene product could not be produced from either a wild type nontranscribed strand or a mutant transcribed strand. If the donated strand is transcribed, however, the spore should be His^+. (b) Tetrads with a $3^+:5^-$ segregation pattern are most easily explained as the result of transfer of a single-strand from a mutant gene to a wild type gene. As for the $5^+:3^-$ tetrads, the sectored colonies would be expected to be derived from either His^+ or His^- spores, depending on whether the transcribed or nontranscribed mutant strand is transferred.

Analysis of data: The proportion of the various classes of sectored colonies (Figure 2) involves a number of different factors, which can be expressed in the following probabilities. The probability that a chromosome with *HIS4* (respectively, *his4*) donates a strand is a (respectively, b). Given that a chromosome with *HIS4* (respectively, *his4*) donates a strand, the conditional probabilities that the strand is transcribed and non-transcribed are c and $1\text{-}c$ (respectively, d

and 1-d). Given that a chromosome with *HIS4* (respectively, *his4*) donates a strand, the conditional probabilities that the mismatch will not be repaired are e for the transcribed strand and f for the nontranscribed strand (respectively, g and h). The respective probabilities of class 1 (nontranscribed strand from *HIS4*), class 2 (transcribed strand from *HIS4*), class 3 (transcribed strand from *his4*), and class 4 (nontranscribed strand from *his4*) tetrads are: $a(1\text{-}c)f$, ace, bdg, and $b(1\text{-}d)h$.

We make the following assumptions: (1) both chromosomes donate with equal probability ($a = b$), (2) the probability that a transcribed strand is donated is the same for the chromosomes containing the wild-type and mutant alleles ($c = d$), and (3) the probability of correction depends only on the individual mismatch ($e = h, f = g$). With these assumptions, the respective probabilities of class 1, class 2, class 3, and class 4 tetrads are: $P(1) = a(1\text{-}c)f$, $P(2) = ace$, $P(3) = acf$, and $P(4) = a(1\text{-}c)e$.

The value c (probability that the transcribed strand is donated and the mismatch is not repaired) can be calculated as

$$c = \frac{P(2) + P(3)}{P(1) + P(2) + P(3) + P(4).}$$

In the numerator and denominator of the above formula, one may use either the probabilities or frequencies of the classes.

The ratio e/f, representing the relative frequencies with which two different mismatches are left uncorrected, is calculated as

$$\frac{e}{f} = \frac{P(2) + P(4)}{P(1) + P(3)}$$

RESULTS

Experimental rationale: The approach to determining whether the transcribed or nontranscribed DNA strand is donated in meiotic recombination events at the *HIS4* locus is indicated in Figure 2. As described in the Introduction, genetic data support the conclusion that heteroduplex formation in yeast is usually asymmetric (FOGEL, MORTIMER and LUSNAK 1981). A tetrad with the 5^+:3^- segregation pattern, therefore, represents a meiosis in which a single DNA strand derived from the wild type allele has been transferred to the chromosome containing the mutant allele, and a 3^+:5^- segregation pattern reflects transfer of a single DNA strand derived from a mutant allele to the chromosome containing the wild type allele. In the 5^+:3^- tetrads, the DNA strand (containing wild type information) that is donated could be either the transcribed or nontranscribed strand (Figure 2a). If the non-transcribed strand is donated, the spore containing the heteroduplex would be unable to grow on medium lacking histidine, since the strand containing the wild type information would not be expressed (class 1). Alternatively, if the wild type transcribed strand is donated, the spore should be phenotypically wild type (class 2). Similarly, for the 3^+:5^- tetrads, a sectored colony derived from a spore that is phenotypically mutant reflects transfer of a transcribed mutant DNA strand (class 3) and a sectored colony derived from a phenotypically wild type spore (class 4) reflects transfer of a nontranscribed mutant strand (Figure 2b).

The same four classes of sectored colonies can also be identified in tetrads with other segregation patterns. For example, 7^+:1^- tetrads (3 His$^+$:1 sectored His$^+$/His$^-$ spore colonies) represent tetrads that have had one conversion and one PMS event (two asymmetric heteroduplexes). The sectored colony in the 7^+:1^- tetrad, therefore, presumably represents a heteroduplex in which a strand was donated from the wild type chromosome to the mutant chromosome (class 1 or class 2) whereas the sectored colony in the 1^+:7^- tetrads represent either class 3 or class 4 events. Aberrant 6^+:2^- tetrads (2 His$^+$:2 sectored His$^+$/His$^-$ spore colonies) represent two unrepaired heteroduplexes in which the wild-type chromosomes donated strands to the mutant chromosomes (class 1 and 2) whereas the aberrant 2^+:6^- tetrads reflect either class 3 or class 4 events. Aberrant 4:4 tetrads (1 His$^+$:1 His$^-$:2 sectored His$^+$/His$^-$ spore colonies) cannot be classified as class 1–4 events, since we cannot determine whether the strands transferred to form the heteroduplex were derived from the mutant or wild-type chromosomes.

In order to detect which DNA strand is transferred to form a heteroduplex by genetic methods, one must classify a spore as wild type or mutant and then allow each classified spore to form a colony under conditions allowing the detection of sectored colonies. Previously, FOGEL and HURST (1967) had shown that yeast spores with a *his1* mutation fail to germinate in medium lacking histidine; in spores derived from a diploid with mutant *his1* heteroalleles, His$^+$ recombinants could be detected by finding spores that could bud in the absence of histidine. In our studies, we analyzed strains that were heterozygous for mutations at the *HIS4* locus that had high levels of PMS. The experimental protocol used involved several steps (Figure 3): (1) tetrads were dissected onto plates containing a thin layer of solid medium lacking histidine, (2) after about 8 hours at 32°, the spores in each tetrad were examined by microscopy to determine whether they had divided; most tetrads had two spores that had divided several times and two spores that had not divided, (3) the layer of agar containing the spores was transferred on top of a layer of solid nonselective (histidine-containing) medium and the cells were incubated until colonies had formed (about 3 days) and (4) the resulting colonies were replica-plated to medium lacking histidine and sectored colonies were scored after about 24 hr of further incubation.

This protocol appears to determine the phenotype of the spore fairly accurately without causing much

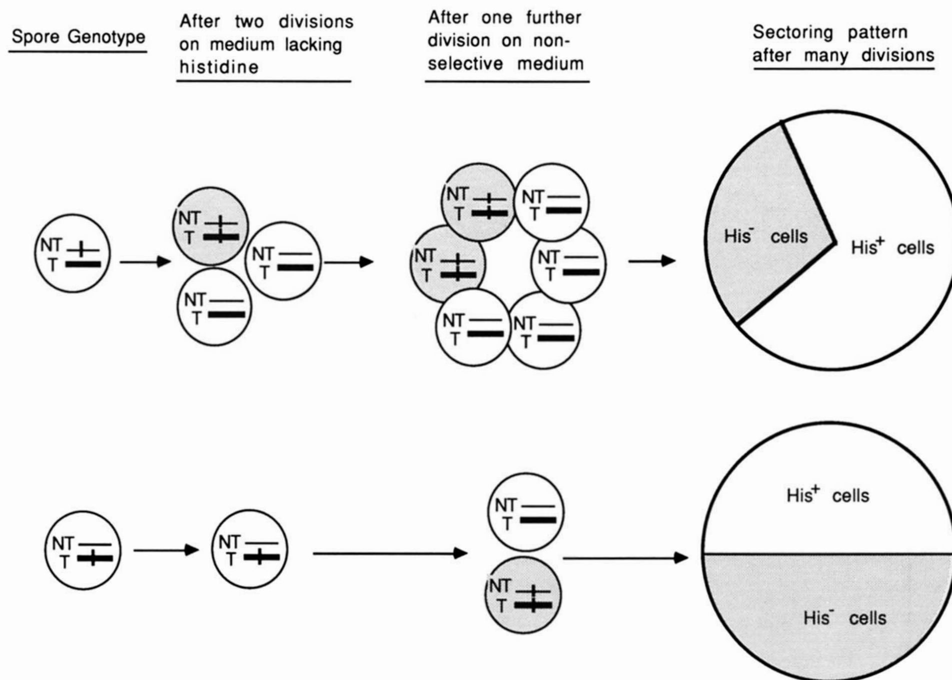

FIGURE 3.—Method for detecting whether sectored colonies are derived from His$^+$ or His$^-$ spores. Spores are indicated by small circles and the DNA strands are indicated by thick (transcribed) and thin (nontranscribed) lines. The mutant base is indicated by short vertical lines. Spores and colonies with the mutant phenotype are shaded and wild-type spores and colonies are not. As described in Figure 2, in tetrads with the 3^+:5^- segregation pattern, a single DNA strand of mutant information is transferred to a wild-type gene. The transferred strand could be the nontranscribed (upper panel) or the transcribed strand (lower panel). To determine which strand is transferred, we determined whether the genotype of the spore is His$^+$ (representing transfer of the nontranscribed mutant strand) or His$^-$ (representing transfer of the transcribed mutant strand) by dissecting spores on medium lacking histidine. After about 9 hr, His$^+$ spores have divided (usually several times) and His$^-$ spores have not. After microscopically scoring the spore phenotype, we transferred the slab of medium containing the spores on top of nonselective medium that had been overlayed with histidine. In this medium, both His$^+$ and His$^-$ cells grow. After several days of nonselective growth, the colonies derived from the spores were replica-plated to medium lacking histidine, in order to detect sectors. This protocol allows classification of sectored colonies into the four classes shown in Figure 2.

TABLE 1

Non-Mendelian segregation at the *HIS4* locus in the strain DNY26

Dissection protocol	Total No. of tetrads	No. of 6:2 (%)	No. of 2:6 (%)	No. of 5:3 (%)	No. of 3:5 (%)	No. of other (%)
Standard[a]	394	18 (5%)	15 (4%)	40 (10%)	37 (9%)	13 (3%)[b]
Experimental	347	12 (3%)	11 (3%)	43 (12%)	37 (11%)	19 (5%)[c]

Tetrads were dissected from the diploid strain DNY26 using two different protocols. The standard protocol involved tetrad dissection on plates containing a rich growth medium; colonies formed on this medium were replica-plated to medium lacking histidine. The experimental protocol involved dissection on plates containing medium lacking histidine (details in MATERIALS AND METHODS). After about 8 hr of incubation, the slab of medium containing the dissected spores was transferred to a plate containing rich growth medium with excess histidine. The cells were incubated until colonies formed; these colonies were then replica-plated to medium lacking histidine. Each column represents a different class of non-Mendelian (non-2:2) segregation. Although yeast has only four spores, the nomenclature of eight-spored fungi is used, since this nomenclature more conveniently describes PMS events.

[a] Data from NAG, WHITE and PETES (1989).

[b] Includes two 7:1, eight aberrant 4:4, one 8:0, and two aberrant 2:6 tetrads.

[c] Includes one 7:1, 11 aberrant 4:4, three aberrant 6:2, one aberrant 2:6, and three tetrads with three or four PMS events. See Table 2.

loss of sectored colonies. The frequency of 5^+:3^- and 3^+:5^- tetrads observed following this protocol is similar to that observed in tetrads dissected onto nonselective medium (Table 1). In addition, since the ratio of 5^+:3^- tetrads to 3^+:5^- tetrads is similar for the two different protocols, there appears to be no selective loss of either of these two classes. Of 1388 spores analyzed, four gave colonies with a phenotype that was different from that predicted by microscopic examination (error rate of about 0.3%): three spores scored as His$^-$ gave rise to His$^+$ colonies, and one spore scored as His$^+$ gave rise to a His$^-$ colony.

Evidence that nontranscribed DNA strand is preferentially transferred to form heteroduplexes at the HIS4 locus: The data are shown in Table 2. The diploid DNY26 is heterozygous for mutations at the *HIS4* locus, a locus which (in our genetic background) has a very high level of non-Mendelian (gene conver-

TABLE 2

Segregation patterns for tetrads with PMS events at the *HIS4* locus

Segregation pattern[a]	His phenotype of PMS spore(s)	Class of sectored colony	No. of Tetrads
$5^+:3^-$	−	1	38
●●◐○	+	2	5
$3^+:5^-$	−	3	9
●◐○○	+	4	28
$7^+:1^-$	−	1	1
●●●◐	+	2	0
$1^+:7^-$	−	3	0
◐○○○	+	4	0
Aberrant $4^+:4^-$	−/−		0
●○◐◐	+/−		10
	+/+		1
Aberrant $6^+:2^-$	−/−	1/1	2
●●◐◐	+/−	2/1	1
	+/+	2/2	0
Aberrant $2^+:6^-$	−/−	3/3	0
○○◐◐	+/−	4/3	0
	+/+	4/4	1
Others[b]			3

Tetrads obtained from dissection using the experimental protocol (see Table 1) and having one or two PMS events per tetrad are shown.

[a] Beneath the numerical representation of the segregation pattern is drawn a schematic representation of spore colonies. The dark color represents wild type (HIS$^+$) cells.

[b] Includes tetrads with three or four PMS events.

sion and PMS) segregation (Nag, White and Petes 1989). The mutant allele in diploid DNY26 (*his4-lopc*), which has a high level of PMS, was constructed by insertion of a 26 bp palindromic sequence into a *Sal*I site located 500 bp from the beginning of the *HIS4* gene (Figure 4). As shown in Table 2, all four classes of single PMS tetrads shown in Figure 2 are observed, indicating that both the transcribed and nontranscribed strands can serve as donors in the formation of the heteroduplex. As described in MATERIALS AND METHODS, the proportion of tetrads in these classes is a function of several variables: the efficiency with which each homologous chromosome acts as donor, the efficiency with which each DNA strand (transcribed or nontranscribed) acts as a donor, and the efficiency of repair of the individual mismatches. For our analysis, we assume that (1) the chromosomes containing wild-type and mutant information donate with equal frequency, (2) the probability that a transcribed strand is donated is the same for both mutant and wild-type chromosomes, and (3) the efficiency of repair of mismatches is dependent only on the individual mismatch. The proportion of heteroduplexes in which the transcribed strand is the donor can be calculated by adding the number of class 2 sectored colonies (6 sectored colonies from $5^+:3^-$, $7^+:1^-$ and aberrant $6^+:2^-$ tetrads that were derived from a His$^+$ spore) to the number of class 3 sectored colonies (9 sectored colonies from $3^+:5^-$, $1^+:7^-$ and aberrant $2^+:6^-$ tetrads derived from a His$^-$ spore) and dividing the sum by the total number (89) of sectored colonies in the 5:3, 3:5, 7:1, 1:7 and aberrant 6:2 and 2:6 tetrads (see MATERIALS AND METHODS). We calculate that the transcribed strand is donated at a frequency of 0.17 and the non-transcribed strand is donated at a frequency of 0.83. In summary, although both strands can be donated to initiate a heteroduplex, the nontranscribed strand is preferentially donated ($\chi^2 = 38$).

Evidence that the efficiency of repair of the palindromic mismatch is independent of the context of the DNA sequences adjacent to the "hairpin": For any nonpalindromic mutation, two different kinds of mismatches would be expected to be present in heteroduplexes. For example, if the mutant nontranscribed strand contains a T (with the wild type substitution in the same strand being an A), then an A/A mismatch would occur for a heteroduplex formed between the mutant nontranscribed and the wild type transcribed strands, and a T/T mismatch for a heteroduplex formed between the mutant transcribed and wild type nontranscribed strands. The number of tetrads in each of the four classes shown in Figure 2 will be affected by two factors: which DNA strand (transcribed or nontranscribed) is transferred, and which mismatch is repaired more efficiently. By the equations described in the MATERIALS AND METHODS, the efficiency of repair (e/f) of different mismatches can be determined *independently* of which DNA strand is transferred.

Since the insertion in *his4-lopc* is palindromic, the DNA sequences in the "hairpin" are the same for all heteroduplexes (Figure 4). The DNA sequences adjacent to the looped-out bases, however, are different. Thus, classes 1 and 3 represent one context and classes 2 and 4 another (Figures 2 and 4). Since the sum of classes 1 and 3 (53) is not significantly ($\chi^2 = 2.8$) different from that of classes 2 and 4 (36), we conclude that the efficiency of repair of the palindromic mismatch is not greatly affected by the bases that are adjacent to the looped-out bases. The observations that the number of 6:2 tetrads approximately equals the number of 2:6 tetrads and the numbers of 5:3 and 3:5 tetrads are approximately equal support the assumption that both chromosomes donate DNA strands with equal efficiency.

DISCUSSION

We find that at the *HIS4* locus, either the transcribed or nontranscribed strand can be a donor in the formation of a heteroduplex. There is, however, a significant bias in favor of transfer of the nontranscribed strand in spores with an unrepaired mis-

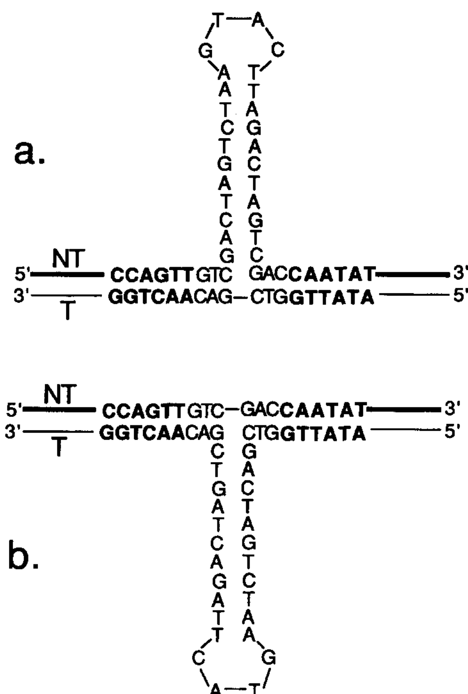

FIGURE 4.—Expected mismatches for heteroduplexes formed between a DNA strand with a palindromic insertion (*his4-lopc*) and a strand with wild type information (*HIS4*). The *his4-lopc* mutation was constructed by inserting a 26 bp oligonucleotide with TCGA "sticky ends" into a *Sal*I restriction site (GTCGAC) of the *HIS4* gene (NAG, WHITE and PETES 1989). Sequences flanking the *Sal*I site are shown in bold-face type. NT and T indicate the nontranscribed and transcribed strands, respectively. Because the *lopc* insertion is palindromic, in a heteroduplex with a wild type gene, base-pairing within the inserted sequences is expected. (a) Heteroduplex formation between a nontranscribed mutant strand and a transcribed wild-type strand. (b) Heteroduplex formation between a transcribed mutant strand and a nontranscribed wild-type strand.

match in a heteroduplex. The simplest interpretation of this result is that the initiation of strand transfer between chromosomes is biased; as discussed below, M. LICHTEN, C. GOYON, A. NICOLAS, N. P. SCHULTES, D. TRECO, J. W. SZOSTAK and J. HABER (personal communication) have observed a similar incomplete bias for the non-transcribed strand at the *ARG4* locus of yeast.

Since we can identify which strand is transferred only in spores with an unrepaired mismatch, the possibility that the observed strand bias is the result of a bias in the mismatch correction system must be examined. One possibility is that mismatched bases are excised preferentially from the transcribed strand regardless of which strand is donated. MELLON and HANAWALT (1989) reported that transcription stimulated removal of pyrimidine dimers from the transcribed strand of the lactose operon of *Escherichia coli*. Since all heteroduplexes with a mismatch have both a transcribed and nontranscribed strand, preferential removal of the mismatched base from the transcribed strand would not produce our observed strand bias.

An alternative type of repair bias is that repair of the mismatch occurs more efficiently when the transcribed strand is donated. Since the frequency of gene conversion for a well-repaired mismatch (*his4-Sal*) at the same position in the *HIS4* gene as *his4-lopc* is 31% (NAG, WHITE and PETES 1989), whereas the frequency of gene conversion for *his4-lopc* is 7%, we can conclude that most (about 80%) of the conversion events at this position are the result of a repair system that is sensitive to the type of mismatch. Our data can be explained in two ways. Our favored explanation is that the bias in strand transfer observed in spores with unrepaired heteroduplexes reflects a bias in strand donation during the initiation of heteroduplex formation and the action of a mismatch repair system that is sensitive only to the type of mismatch. The alternative (and more complicated) possibility is that the transcribed and nontranscribed strands are donated with equal frequency, but there is a mismatch repair system that is sensitive to both the mismatch *and* the strand that is transferred, repairing heteroduplexes in which the transcribed strand is donated more efficiently. Below, we will discuss the strand bias as reflecting the efficiency of strand transfer at the initiation of heteroduplex formation, although we cannot exclude the alternative possibility.

The location of the site (or sites) required to initiate heteroduplex formation at the *HIS4* locus is not known. Mutant alleles at the 5′ end of the *HIS4* gene convert at frequencies that are about two-fold higher than those at the 3′ end (M. WHITE, P. DETLOFF, M. STRAND and T. PETES, unpublished data). Such polarity gradients are usually interpreted as indicating that heteroduplex formation is initiated near the 5′ end of the gene and heteroduplexes are propagated to different extents into the gene (HOLLIDAY 1964). In the context of the Radding model (Figure 1a), the biased strand transfer may be interpreted as indicating that, although either strand at the initiation site can be nicked to initiate heteroduplex formation, the nontranscribed strand is more frequently nicked than the transcribed strand (allowing more frequent invasion by the nontranscribed strand of the other chromosome). Alternatively, the two strands may be nicked with equal frequency but heteroduplex formation may be propagated preferentially in one direction (5′ to 3′) from the initiation site. By the double-strand break model (Figure 1b), the result suggests that the transcribed strand of the broken chromosome is preferentially degraded, exposing a single-stranded nontranscribed strand that subsequently invades the unbroken chromosome and forms the heteroduplex.

We do not know whether the bias in favor of the nontranscribed strand observed at the *HIS4* and *ARG4* loci is directly related to transcription. It is possible that the bias reflects sequence-specific initiation or processing of recombination events that is independ-

ent of transcription. One argument that transcription is not involved in determining the strand bias is that the rate of meiotic gene conversion at *HIS4* is only slightly affected by promoter deletions that reduce transcription 15-fold (M. WHITE, P. DETLOFF, M. STRAND and T. PETES, unpublished data).

The observed bias in favor of the non-transcribed strand was incomplete. One possibility is that the initiation event that causes the bias recognizes both strands (albeit with different efficiencies). Alternatively, strand transfer initiated from a single site could have a very strong bias, but heteroduplex formation involving the *his4-lopc* insertion could be initiated from multiple sites. We have found that mutations located closer to the 5′ end of the *HIS4* gene (about 500 bp away) have about a 2–3-fold bias (P. DETLOFF and T. PETES, unpublished data), indicating that the degree of bias may be a function of the position of the mutation being examined. In this same study, we used the methods described above to examine the repair of individual mismatches, concluding that C/C mismatches were poorly repaired relative to all others.

As discussed previously, the rate of non-Mendelian segregation at the *HIS4* locus in our genetic background is exceptionally high (about 40%) relative to that reported previously for other loci in *S. cerevisiae*. This high rate of non-Mendelian segregation is specific for this locus; for example, the rate of gene conversion at the *LEU2* locus (located on the same chromosome as *HIS4*) is only 3.6%. We find 2.6% (19 of 741) of the tetrads derived from strains DNY26 have the aberrant 4:4 segregation pattern (Tables 1 and 2), whereas in a study of PMS at the *arg4-16* locus, FOGEL, MORTIMER and LUSNAK (1981) found 0.03% aberrant 4:4's (2.6% 5:3 and 3:5 tetrads). The aberrant 4:4 pattern is interpretable as representing either two unrepaired mismatches in a symmetric heteroduplex or unrepaired mismatches in two independently formed asymmetric heteroduplexes. We favor the latter possibility for three reasons. First, the frequency (0.03) of tetrads with two PMS events (aberrant 4:4, aberrant 6:2 and aberrant 2:6) is approximately that expected as the result of two independent single PMS events ($(0.21)^2$ or 0.04). Second, the observed frequency of aberrant 4:4 events is at least 3-fold lower than the frequency expected (assuming symmetrical heteroduplexes) from the frequency of 6:2, 2:6, 5:3, and 3:5 tetrads (calculated according to equations in FOGEL, MORTIMER and LUSNAK (1981)). Third, some of the observed classes of tetrads, such as the aberrant 6:2 and aberrant 2:6 classes, must represent two independent heteroduplexes rather than a single symmetric heteroduplex.

M. LICHTEN, C. GOYON, A. NICOLAS, N. P. SCHULTES, D. TRECO, J. W. SZOSTAK and J. HABER (personal communication) used denaturing agarose gels to detect physically mismatches at the *ARG4* locus. For a G to C mutation in the initiating ATG codon, they found that C/C mismatches were repaired at least 5-fold less efficiently than G/G mismatches. In addition, in this strain, 3:5 tetrads were significantly (about 4-fold) in excess of 5:3 tetrads. If the PMS events are due to unrepaired C/C mismatches and both chromosomes act as donors equally frequently, these results indicate that the C/C mismatches resulting from transfer of the nontranscribed strand are more frequent than those resulting from transfer of the transcribed strand. These conclusions are in reasonably good agreement with ours.

In summary, in this paper, we present a general genetic method of determining which DNA strand is transferred during *in vivo* formation of a heteroduplex. This method should be applicable to any allele that has a high level of PMS in any gene in which a mutation prevents spore growth (in spores derived from a heterozygous diploid). We conclude from our analysis that, at the *HIS4* locus, although either DNA strand can be donated, the nontranscribed strand is donated more frequently than the transcribed strand.

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