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Genetics

Detection of heteroduplex DNA molecules among the products of *Saccharomyces cerevisiae* meiosis

(postmeiotic segregation/mismatch repair/preferential strand transfer/denaturant-gel electrophoresis/yeast)

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ABSTRACT **We have used denaturant-gel electrophoresis to provide a physical demonstration of heteroduplex DNA in the products of yeast meiosis. We examined heteroduplex formation at *arg4-nsp*, a G·C → C·G transversion that displays a moderately high level of postmeiotic segregation. Of the two possible *arg4-nsp/ARG4* mismatches (G·G and C·C), only C·C was detected in spores from mismatch repair-competent (Pms1$^+$) diploids. In contrast, C·C and G·G were present at nearly equal levels in spores from Pms1$^-$ diploids. These results confirm previous suggestions that postmeiotic segregation spores contain heteroduplex DNA at the site of the marker in question, that C·C is repaired less frequently than is G·G, and that the *PMS1* gene product plays a role in mismatch correction. Combined with the observation that Pms1$^+$ *ARG4/arg4-nsp* diploids produce 3 times more 3$^+$:5^m (wildtype:mutant) tetrads (+, +, +/*m*, *m*) than 5$^+$:3^m tetrads (+, +/*m*, *m*, *m*), these results indicate that, during meiosis, formation of heteroduplex DNA at *ARG4* involves preferential transfer of the sense (nontranscribed) strand of the DNA duplex.**

Central to current models of generalized recombination is an early step in which a single strand of DNA is transferred from one parental duplex to the other, forming a region of heteroduplex DNA (1). Such a structure ensures that the breakage and joining reactions of recombination occur with fidelity and provides an attractive way to search for homology when initiating meiotic chromosome pairing (2).

In the yeast *Saccharomyces cerevisiae*, evidence for formation of heteroduplex DNA during meiosis has largely been provided by genetic examination of tetrads. Although diploids heterozygous for a mutation (*m*/+) usually yield two mutant (*m*) and two wild-type (+) spores, occasionally gene conversion tetrads are produced. These tetrads contain parental alleles in a non-Mendelian ratio, most commonly 6$^+$:2^m or 2$^+$:6^m (numbers refer to the eight single DNA strands in the four spores). Occasionally, postmeiotic segregation tetrads are observed. These contain a haploid spore that, upon outgrowth, segregates both parental alleles at a locus and most often take 3$^+$:5^m and 5$^+$:3^m (+, +/*m*, *m*, *m* and +, +, +/*m*, *m*, respectively) segregation patterns (3). For most mutations, postmeiotic segregation forms a minor fraction of total gene conversions; however, alleles that show high levels of postmeiotic segregation have been isolated (3–6). Several are G·C → C·G transversions (refs. 4 and 5; P. Detloff, D. Nag, and T. Petes, personal communication).

Postmeiotic segregation shares properties with other types of gene conversion, including association with crossing-over of flanking markers (3). For this reason, it has been suggested that postmeiotic segregation and 6$^+$:2^m/2$^+$:6^m segregations are alternate outcomes of a recombination event that forms a joint structure with heteroduplex DNA on at least one of the two participating chromosomes (3, 7, 8). Correction of a mismatch in heteroduplex DNA would result in either 6$^+$:2^m/2$^+$:6^m segregations or restoration to the original (4:4) allele ratios; mismatches escaping correction would be detected as postmeiotic segregation. In particular, high-postmeiotic-segregation allele mismatches would be corrected less frequently than low-postmeiotic segregation allele mismatches.

This suggestion is supported by the observation that, during mitosis, different base pair mismatches are corrected with different efficiencies, with mismatches expected for high-postmeiotic segregation mutations (for example, C·C) most frequently escaping repair (9, 10). The product of the *PMS1* gene plays an important role in this mismatch repair. In *pms1* mutant strains, many low-postmeiotic segregation alleles display increased levels of postmeiotic segregation (11), and all base pair mismatches escape mitotic repair at a high frequency (9, 10, 12). Whether all non-postmeiotic segregation gene conversion derives from correction of mismatches in heteroduplex is uncertain. In alternate models, some gene convertants are produced by repair of double-strand breaks or gaps (13, 14).

In this paper, we present the results of experiments directed at understanding the molecular nature of recombination events that occur during meiosis. We have studied the meiotic behavior of *arg4-nsp*, a G·C → C·G transversion located at position +3 in the *ARG4* coding region. Our results indicate that the two mismatches possible at *arg4-nsp* (C·C and G·G) are repaired at very different efficiencies, that C·C mismatches formed during meiosis frequently escape correction, and that a *PMS1*-dependent correction system is responsible for this difference in repair levels.

The formation of heteroduplex DNA on a chromatid involves the replacement of one strand of the duplex with a single strand from another chromatid. For the sake of simplicity, we refer to this entire process as "strand transfer," and refer to the two strands of the duplex at *ARG4* as the "sense" and "antisense" strands; the sense strand is also referred to as the "nontranscribed" strand. We present evidence that, during formation of heteroduplex at *ARG4*, the single strand of DNA replaced by a strand from another chromosome is predominantly the sense strand. Similar conclusions have been drawn by Petes and co-workers for the *HIS4* locus (P. Detloff, D. Nag, and T. Petes, personal communication).

MATERIALS AND METHODS

Strains. Relevant genotypes of the yeast strains used in this work are as follows: MGD409, *arg4-nsp/ARG4 PMS1/PMS1*;

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ORD002, *arg4-nsp/ARG4 PMS1/PMS1*; MJL332, *ARG4/ARG4 PMS1/PMS1*; MJL334, *arg4-nsp/arg4-nsp PMS1/PMS1*; MJL358, *arg4-nsp/ARG4 pms1Δ::URA3/pms1Δ::URA3*; MJL361, *ARG4/ARG4 pms1Δ::URA3/pms1Δ::URA3*; MJL362, *arg4-nsp/arg4-nsp pms1Δ::URA3/pms1Δ::URA3*. *arg4-nsp* is a G·C → C·G transversion located at position +3 in the *ARG4* coding region. MGD409 was constructed by mating an *arg4-nsp* derivative of MGA1 with a derivative of MGA3 (see ref. 5 for full genotype and details of construction). Meiotic segregants of MGD409 were backcrossed to the appropriate parent to yield MJL332 and MJL334. ORD002 is closely related to MGD409; it contains a 1.2-kilobase (kb) *Hin*dIII *URA3* fragment (15) and a 1.5-kb *Eco*RI *TRP1* fragment (16) inserted 1.8 kb upstream and 2.4 kb downstream of the *arg4-nsp* site, respectively. *pms1Δ::URA3* strains were constructed by replacing *PMS1* sequences between an *Mlu* I and an *Nco* I site with the *URA3* *Hin*dIII fragment, removing 881 amino acids of *PMS1* (17). The following diploid pairs are isogenic except at *PMS1*: MDG409 and MJL358, MJL332 and MJL361, and MJL334 and MJL362.

Plasmids. pNPS500 contains a 3.3-kb *Pst* I *ARG4* gene fragment (18) inserted at the *Pst* I site of pMLC28. pNPS425 contains an *arg4-nsp* mutant 3.3-kb *Pst* I fragment inserted at the *Pst* I site of pMLC12. pMLC12 and pMLC28 are closely related derivatives of pSDC12 and were a gift from Brian Seed (19).

Artificial Heteroduplex Mixtures. Plasmids pNPS425 and pNPS500 were linearized with *Bam*HI and mixed at 0.05 μg/ml each in TE buffer containing 10 mM NaCl (TE = 10 mM Tris·HCl/1 mM Na_2EDTA, pH 8.0). The mixture was heated at 90°C for 2 min, mixed with an equal volume of TE containing 200 mM NaCl at 90°C, and incubated at 65°C for 3 hr. DNA was concentrated by ethanol precipitation and stored in TE buffer.

Sporulation and Isolation of DNA. Diploids were sporulated as described (5). Spores were purified by two centrifugations at 15,000 × *g* in a swinging bucket rotor (first through 70%, then 66% Percoll) and resuspended in 100 mM Tris·HCl/10 mM Na_2EDTA/2% sodium dodecyl sulfate (SDS), pH 8.0. An equal volume of phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol), was added, and acid-washed and siliconized 0.5-mm glass beads (Sigma) were added to the organic/aqueous interface. Tubes were agitated on a Vortex mixer for 2 min and centrifuged 5 min at 5000 × *g*. The aqueous layer was extracted with chloroform, NaCl was added to 0.2 M, and DNA was precipitated with an equal volume of isopropyl alcohol. The pellet was resuspended in TE containing 40 μg of RNase per ml, and the mixture was incubated 1 hr at 37°C and processed through Qiagen (Qiagen, Studio City, CA) resin as recommended. DNA was isolated from mitotic cells as described (20).

Gel Electrophoresis. Gels were run at 60°C and 150 V in a SE600 vertical gel apparatus (Hoefer). Temperature was maintained by circulating hot water through buffer cooling coils. Buffers and denaturant mixture were as described (21). Gels in Figs. 1 and 2 contained 6.5% polyacrylamide (37.5:1 monomer/bisacrylamide) and in Fig. 3 contained 11% polyacrylamide (49:1 monomer/bisacrylamide). Gels were dried (see Fig. 1) or transferred to Nytran (Schleicher & Schuell) by electroblotting. Membranes were placed on Whatman 3MM paper saturated with 0.5 M NaOH/1.5 M NaCl for 10 min, on a 3MM paper saturated with 2× SSPE (22) for 5 min, air-dried, and baked 1–2 hr at 80°C.

Hybridization. Filters probed with oligonucleotides were prehybridized and hybridized with probes at 42°C in 6× SSPE/1% SDS/0.05% nonfat dried milk/500 μg of denatured carrier DNA per ml and were washed three times for 20 min at 42°C in 2× SSPE/0.1% SDS. Filters probed with hexamer-primed *ARG4 Sac* I–*Eco*RV fragment were prehybridized and hybridized as above, but at 65°C with 3.1× SSPE, and were washed twice at room temperature in 2× SSPE, once at 60°C in 0.4× SSPE, and twice at 60°C in 0.1× SSPE. All washes were for 15 min.

Quantitation. The amount of material present at heteroduplex positions was determined by comparison of band density with the density of standards (pNPS425 digested with *Sac* I) added to sample lanes (see Fig. 3, lanes 8–10). The amount present at the homoduplex position was similarly determined by using lower dilutions of the same plasmid digest (data not shown). Both a Zeineh integrating scanning densitometer (LKB) and video densitometry were used.

RESULTS

Tetrads from *PMS1/PMS1* strains heterozygous for *arg4-nsp* display a moderately high level of postmeiotic segregation (Table 1). A significant disparity in recovery of $3^+:5^m$ versus $5^+:3^m$ tetrads was observed ($P > 0.95$; Fisher's exact test); about 3 in 4 postmeiotic segregation tetrads displayed a $3^+:5^m$ pattern. No such dramatic disparity was observed in tetrads from the *pms1/pms1* diploid MJL362. A similar *PMS1*-dependent disparity in $3^+:5^m$ versus $5^+:3^m$ tetrads is observed for *arg4-16* (3, 11), a G·C → C·G transversion 338 bp downstream from *arg4-nsp* (4).

About 2% of tetrads from MGD409, the strain used for physical examination of spore DNA, contained an *arg4-nsp/ARG4* spore. This level of postmeiotic segregation corresponds to about one *arg4-nsp/ARG4* mismatch per 200 copies of the *ARG4* gene (0.5%).

Both Forms of an *arg4-nsp/ARG4* Mismatch Can Be Detected. We used a modification of the denaturing gradient gel electrophoresis technique (21) to resolve *arg4-nsp/ARG4* molecules from homoduplex molecules. In this technique, restriction enzyme-digested DNA is displayed on polyacrylamide gels run in conditions that approach the melting temperature of the fragment of interest. At the appropriate effective temperature, mismatch-bearing heteroduplex DNA molecules are selectively retarded relative to parental homoduplexes (23).

An example of this effect on heteroduplex molecules that contain an *arg4-nsp/ARG4* mismatch is shown in Fig. 1. An artificial heteroduplex mixture of *ARG4/ARG4*, *arg4-nsp/arg4-nsp*, and *ARG4/arg4-nsp* fragments was displayed on perpendicular denaturant-gradient gels. Two species were resolved from the homoduplex species. These two species were not observed in gels loaded with homoduplex fragments (data not shown); therefore, they contain heteroduplex molecules with a C·C or a G·G mismatch at the site of *arg4-nsp*.

The mismatch contained in the two heteroduplex species was determined by displaying the heteroduplex mixture on a uniform denaturant gel and hybridizing gel contents with oligonucleotides corresponding to either the sense or antisense strand of *ARG4* or *arg4-nsp* (Fig. 2). Annealing conditions used required complete homology for efficient hybridization. The slower-migrating heteroduplex species hybridized to both the *arg4-nsp* antisense strand and the *ARG4* sense strand; therefore, it contains a C·C mismatch. Similarly, the more rapidly-migrating species was identified as containing a G·G mismatched base pair. Thus, under appro-

Table 1. Meiotic segregation of *arg4-nsp*

Strain	4:4	$6^+:2^m$	$2^+:6^m$	$5^+:3^m$	$3^+:5^m$	ab4:4	$7^+:1^m$
MGD409 (*PMS1*)*	914	49	40	4	16	1	0
ORD002 (*PMS1*)	792	67	23	2	5	0	0
Total *PMS1*	1706	116	63	6	21	1	0
MJL362 (*pms1Δ*)	138	3	5	6	3	1	1

Segregation patterns refer to each of the eight DNA single strands in the four spores.

*Includes data from 530 tetrads previously reported (5).

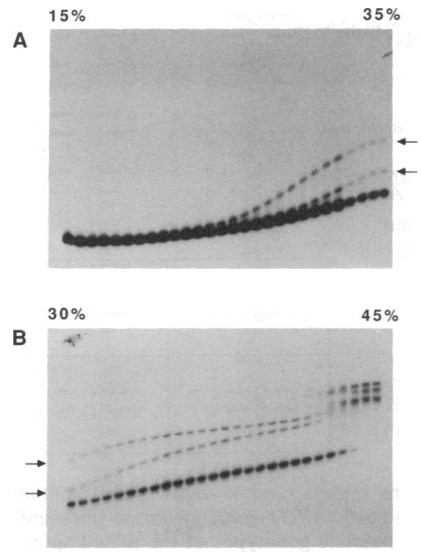

FIG. 1. Detection of heteroduplex DNA molecules. A 298-base-pair *Sac* I–*Eco*RV fragment containing the site of *arg4-nsp* (−38 to +260 relative to the *ARG4* translation start site) was gel-purified from an artificial heteroduplex mixture of pNPS500 (*ARG4*) and pNPS425 (*arg4-nsp*), end-labeled with T4 DNA polymerase (22), and displayed on gels containing a linear gradient of denaturant [100% = 7 M urea/40% (wt/vol) formamide] perpendicular to the direction of electrophoresis. Denaturant concentrations at the ends of two separate gels (*A* and *B*) are indicated. Arrows indicate mismatch-containing fragments. *ARG4/ARG4* and *arg4-nsp/arg4-nsp* homoduplexes are not resolved from each other.

priate conditions, the C·C and G·G mismatch forms of *arg4-nsp/ARG4* can be resolved both from one another and from the two parental homoduplexes.

C·C Mismatches Survive Correction During Meiosis. This technique was used to examine spore DNA for *arg4-nsp/ARG4* mismatches. Digests of spore DNA were displayed on denaturant gels, transferred to filters, and hybridized with radiolabeled *ARG4* DNA (Fig. 3). A signal at the C·C position was present in spore DNA from the heterozygous (*ARG4/arg4-nsp*) diploid MGD409 (Fig. 3*A*, lane 3). A comparison of this signal to internal standards (Fig. 3*A*, lanes 8–10) indicated that C·C mismatches were present in 0.7% ± 0.3% of copies of the *ARG4* gene, in agreement with the value (0.5%) predicted from levels of postmeiotic segregation observed in this strain (see Table 1).

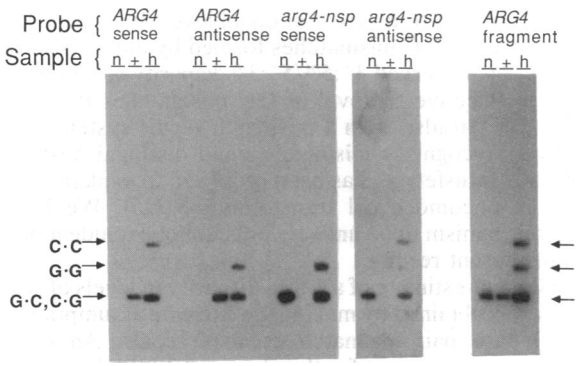

FIG. 2. Determination of the mismatch contained in the two heteroduplex species. *Sac* I/*Eco*RV-digested samples were displayed on a 35% denaturant gel, and gel contents were transferred to Nytran. Sets of lanes were hybridized with end-labeled oligonucleotides (22) or with the hexamer primer-labeled (24) *Sac* I–*Eco*RV fragment. Oligonucleotides used as probes were the *ARG4* sense strand, CAAACATGTCAGACG; *ARG4* antisense strand, CGTCTGACATGTTTG; *arg4-nsp* sense strand, CAAACATCTCAGACG; and *arg4-nsp* antisense strand, CGTCTGAGATGTTTG. Boldface letters indicate the nucleotide at the position of *arg4-nsp*. Samples: n, pNPS425 (*arg4-nsp*); +, pNPS500 (*ARG4*); h, artificial heteroduplex mixture of pNPS425 and pNPS500.

This signal was absent from control samples containing DNA from mitotic cells (Fig. 3*A*, lane 2) or spore DNA from homozygous diploids (Fig. 3*A*, lanes 4–6). In addition, no signal was detected at the G·G position in all samples. The lower limit of reliable detection in these experiments is about 0.1% of *ARG4* copies (Fig. 3*A*, lane 8; and data not shown). Therefore, C·C is at least 5 times as common as G·G in spores from *arg4-nsp/ARG4* diploids. It is likely that most, if not all, postmeiotic segregation spores contained a C·C base pair mismatch at *arg4-nsp*.

One way to account for the differential recovery of C·C versus G·G base pair mismatches is to suggest that both are formed during meiosis, but that only C·C mismatches escape correction. In *S. cerevisiae*, correction of most base pair mismatches is thought to depend on the *PMS1* gene product (9–12). Spores from a *pms1*Δ::*URA3*-homozygous diploid contained both C·C and G·G mismatches; signals of nearly equal intensities were observed at the two heteroduplex positions (Fig. 3*B*). The signal at the C·C position in DNA from *pms1*Δ/*pms1*Δ spores was somewhat greater (1.6 ± 0.2-fold) than the signal at the C·C position in DNA from *PMS1/PMS1* spores (data not shown). Therefore, removal of

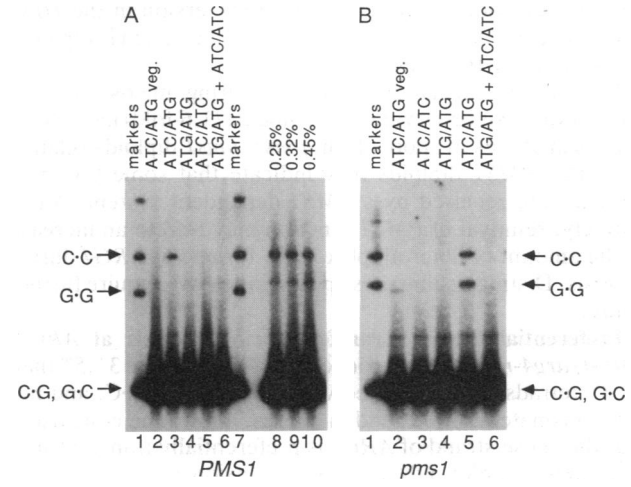

FIG. 3. C·C mismatches escape correction during meiosis. *Sac* I/*Eco*RV digests of spore DNA were displayed on a uniform-denaturant gel, transferred to nylon, and hybridized with radiolabeled *ARG4 Sac* I–*Eco*RV fragment. Marker lanes (1 and 7 in *A* and 1 in *B*) contain a *Sac* I/*Eco*RV digest of an artificial heteroduplex mixture. Arrows indicate the positions of C·C or G·G mismatch-containing fragments and the position of homoduplex fragments. (*A*) *PMS1/PMS1* strains. Lanes 2–6 contain about 1 μg of a *Sac* I/*Eco*RV digest of DNA isolated from mitotic cells of MGD409 (*arg4-nsp/ARG4*) (lane 2), spores from MGD409 (*arg4-nsp/ARG4*) (lane 3), spores from MJL332 (*ARG4/ARG4*) (lane 4), spores from MJL334 (*arg4-nsp/arg4-nsp*) (lane 5), and a mixture of spores from MJL332 and MJL334 (lane 6). Lanes 8–10 contain 1 μg of a *Sac* I/*Eco*RV digest of spore DNA from MGD409 mixed with different amounts of a *Sac* I digest on pNPS425. The signal at the *Sac* I–*Sac* I fragment position (just above G·G) was used for quantitation. Figures above lanes refer to signal intensity of the *Sac* I–*Sac* I fragment relative to that of the homoduplex *Sac* I–*Eco*RV fragment. The *Sac* I digest of pNPS425 was used at 0.5 pg (2.5×10^{-3} copies per haploid genome) in lane 8, at 0.7 pg (3.2×10^{-3} copies per haploid genome) in lane 9, and at 0.9 pg (4.5×10^{-3} copies per haploid genome) in lane 10. (*B*) *pms1/pms1* strains. Lanes 2–6 contain 1 μg of a *Sac* I/*Eco*RV digest of DNA from mitotic cells of MGD409 (*arg4-nsp/ARG4*) (lane 2), spores from MJL358 (*arg4-nsp/arg4-nsp*, *pms1/pms1*) (lane 3), spores from MJL361 (*ARG4/ARG4*, *pms1/pms1*) (lane 4), spores from MJL362 (*arg4-nsp/ARG4*, *pms1/pms1*) (lane 5), and a mixture of spores from MJL358 and MJL361 (lane 6).

PMS1 gene function results in a modest increase in recovery of C·C mismatches and a substantial increase in recovery of G·G.

DISCUSSION

We have used denaturing gel electrophoresis to demonstrate the presence of heteroduplex DNA in spores produced by a diploid heterozygous for a G·C → C·G mutation, *arg4-nsp*. DNA molecules bearing a C·C base pair mismatch were detected in spores from *PMS1/PMS1* strains at levels consistent with the frequency of postmeiotic segregation for *arg4-nsp*; G·G mismatches were not observed. Spores from *pms1/pms1* diploids contained C·C and G·G base pair mismatches at approximately equal levels. These results are most simply interpreted by assuming that C·C and G·G mismatches are formed at equal levels during meiosis and that G·G mismatches are efficiently corrected by a *PMS1*-dependent system, while a significant fraction of C·C mismatches are not. Our results indicate that C·C escapes correction at least 5 times more frequently than does G·G.

This conclusion is supported by studies of mitotic mismatch repair in *S. cerevisiae* (9, 10). C·C mismatches were observed to escape correction at least 4.5 times more frequently than G·G; a *PMS1*-dependent repair system is responsible for this disparity. In addition, P. Detloff, D. Nag, and T. Petes (personal communication) have examined the meiotic behavior of a G·C → C·G transversion in the *HIS4* gene and conclude that the C·C base pair mismatch is repaired less efficiently than G·G.

Do all C·C mismatches formed during meiosis escape correction? Our observation of a modest (1.6-fold) increase in C·C mismatches in spores from *pms1/pms1* diploids relative to *PMS1/PMS1* diploids may indicate that some C·C mismatches are repaired by a *PMS1*-dependent system. Alternatively, removal of *PMS1* function may lead to an increase in the amount of heteroduplex DNA formed at *ARG4* during meiosis. Distinguishing these possibilities will require further study.

Preferential Strand Transfer During Meiosis at *ARG4*. *ARG4/arg4-nsp* strains produce three times more $3^+:5^m$ than $5^+:3^m$ tetrads. This, coupled with the fact that C·C, and not G·G, mismatches are found in spores, allows the conclusion that the sense strand of *ARG4* is preferentially transferred to form heteroduplex DNA. This conclusion is based on two assumptions: first, that strand transfer is asymmetric; second, that all C·C mismatches enjoy an equal probability of survival. Our argument, presented below, is summarized in Fig. 4; the validity of these two assumptions will be discussed later.

If strand transfer is asymmetric, then a $3^+:5^m$ tetrad most likely is produced by replacement of a strand on an *ARG4* chromatid with a single strand of *arg4-nsp* DNA. When the sense strand is transferred, the resulting heteroduplex will contain a C·C mismatch; transfer of the antisense strand produces a G·G mismatch. Conversely, in a $5^+:3^m$ tetrad, an *ARG4*-marked strand is inserted in an *arg4-nsp* chromatid. Transfer of the sense strand yields G·G; transfer of the antisense strand yields C·C. Since a *PMS1*-dependent correction system efficiently removes G·G mismatches, most postmeiotic segregants arise from spores that contain C·C mismatches. Therefore, most $3^+:5^m$ tetrads are produced by transfer of the sense strand and most $5^+:3^m$ tetrads are produced by antisense strand transfer.

By this argument, the ratio of frequencies of $3^+:5^m$ and $5^+:3^m$ tetrads provides a measure of the relative levels of sense-strand and antisense-strand transfer. Since a 3-fold excess of $3^+:5^m$ tetrads was observed, the sense strand of *ARG4* was transferred to form a heteroduplex at least 3 times more often than the antisense strand.

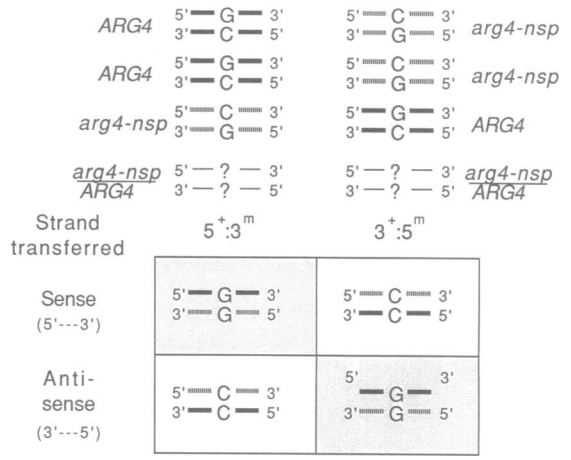

FIG. 4. The sense strand of *ARG4* is preferentially transferred. Solid lines indicate *ARG4* strands; dotted lines indicate *arg4-nsp* strands. The possible genotypes of the mixed spore in a $5^+:3^m$ or $3^+:5^m$ tetrad are shown in the boxed matrix. Shaded boxes contain mismatches not found in spores (presumably removed by *PMS1*-dependent repair). Since $5^+:3^m$ tetrads are mostly the products of antisense strand transfer, and $3^+:5^m$ tetrads are mostly the products of sense strand transfer, the ratio of the two tetrad types reflects the ratio of antisense/sense strand transfer.

As mentioned above, we assume that strand transfer is asymmetric and that all C·C mismatches survive with equal probability. An alternate explanation would have a heteroduplex formed by symmetrical strand exchange (7). One chromatid would contain a G·G mismatch at *arg4-nsp*; and another, C·C. If C·C escapes correction and if the G·G mismatch is corrected asymmetrically, with the G in the sense strand being preferentially replaced by C, the observed $3^+:5^m/5^+:3^m$ disparity would result. We believe this explanation to be incorrect. In the absence of mismatch correction, symmetrical heteroduplex at *arg4-nsp* would yield a tetrad containing two postmeiotic segregation spores (ab4:4). Such tetrads are not prominent among tetrads from the *pms1/pms1* strain used in this work (see Table 1). Comparisons of levels of ab4:4, $5^+:3^m$ and $3^+:5^m$ tetrads for high-postmeiotic segregation mutations have led others to also conclude that strand transfer in *S. cerevisiae* is usually asymmetric (3, 6).

Another explanation would suggest that sense and antisense strands are transferred at equal levels but that a repair system corrects C·C mismatches formed by antisense strand transfer. The observed $3^+:5^m/5^+:3^m$ disparity would be produced by selective removal of C·C mismatches that would yield $5^+:3^m$ tetrads. Such a mismatch repair system, which selectively recognizes mismatches and distinguishes which strand was transferred, has been proposed to explain marker effects in pneumococcal transformation (25). We believe such a mechanism to be unlikely but cannot exclude it on the basis of present results.

Finally, our estimate of a 3-fold disparity in levels of strand transfer was obtained by making the extreme assumption that no G·G base pair mismatch escapes repair. An equally extreme way to account for the observed 3-fold difference in levels of $3^+:5^m$ and $5^+:3^m$ tetrads would be to suggest that heteroduplex is formed at *ARG4* exclusively by transfer of the sense strand. In this case, the $3^+:5^m/5^+:3^m$ ratio would directly reflect the ratio of the frequency at which C·C and G·G mismatches escape correction. It is likely that neither explanation is completely correct, but rather that heteroduplex is occasionally formed by transfer of the antisense strand, and that occasionally G·G mismatches survive to yield postmeiotic segregants.

arg4-16, another G·C → C·G mutation, also displays $3^+:5^m$ and $5^+:3^m$ segregations in a 3:1 ratio (ref. 3; N.P.S. and

J.W.S., unpublished data). This provides further evidence for a disparity in strand transfer at *ARG4* favoring the sense strand, since it is likely that C·C and G·G mismatches at *arg4-16* are corrected in a manner similar to those at *arg4-nsp* (10). P. Detloff, D. Nag, and T. Petes (personal communication) examined the meiotic behavior of two mutations in the *HIS4* gene and concluded that the sense strand of *HIS4* is also preferentially transferred. In summary, examinations of heteroduplex formation with four mutations in two different genes are all consistent with the conclusion that the sense strand is preferentially transferred during meiosis.

Implications for Models of Meiotic Recombination. If a recombination mechanism is to result in preferential strand transfer, it must incorporate directionality and strand specificity in at least one step. A model that meets both of these requirements is diagrammed in Fig. 5. Here, strand transfer disparity at *arg4-nsp* is produced by initiation via a site-specific double-strand break upstream of *arg4-nsp* and 5′–3′ recision to create single strands that later will form heteroduplex. As drawn, this model predicts that only the sense strand will be transferred. However, transfer of information from the antisense strand can be accommodated by suggesting either that some initiating breaks are downstream of *arg4-nsp* or that some breaks are processed to form single-strand overhangs with free 5′ ends.

Several observations are consistent with this mechanism. Sun *et al.* (26) and Cao *et al.* (27) have presented evidence for meiosis-specific double-strand breaks in sequences associated with high levels of meiotic recombination. One of these breaks occurs in a region upstream of *ARG4* that Nicolas *et al.* identify as a site for initiation of meiotic gene conversion (5, 26). Finally, single-strand tails with a free 3′ end are associated with breaks created by the *HO* endonuclease at the *MAT* locus (28) and with the meiosis-specific break upstream of *ARG4* (Hong Sun, D.T., and J.W.S., unpublished data).

Of course, the mechanism in Fig. 5 does not include all possible ways to account for preferential strand transfer. For example, strand-transfer disparity can also be produced by using a site- and strand-specific single-strand gap as an initiating lesion (8) or by mechanisms that invoke strand specificity in invasion of the duplex by single strands and site specificity in the resolution of intermediates. Distinguishing between these many possible explanations will ultimately require a characterization of intermediates in meiotic recombination. We expect that the ability to detect heteroduplex DNA molecules directly will play an important role in such investigations.

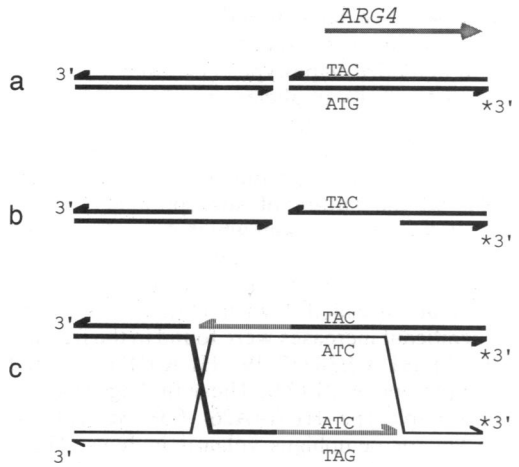

FIG. 5. A mechanism for initiation of meiotic recombination resulting in strand transfer disparity. The model presented here is derived from those of others (13, 14). Arrowheads indicate 3′ ends; the *ARG4* sense strand is starred. (*a*) A site-specific double-strand break occurs upstream of *ARG4*. (*b*) 5′–3′ excision produces single strands with a free 3′ end. The strand removed at the site of *arg4-nsp* is the sense strand. (*c*) The single strands invade another chromatid, displacing the sense strand. Repair synthesis (dashed lines) fills gaps. Information on the sense strand is donated to form heteroduplex DNA. See Szostak *et al.* (14) for further repair and resolution steps.

Note Added in Proof. A significant portion of the results cited as a personal communication from P. Detloff, D. Nag, and T. Petes has appeared recently (29).

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1. Hastings, P. J. (1987) in *Meiosis*, ed. Moens, P. B. (Academic, Orlando, FL), pp. 139–156.
2. Carpenter, A. T. C. (1987) *BioEssays* **6**, 232–236.
3. Fogel, S., Mortimer, R. K. & Lusnak, K. (1981) in *The Molecular Biology of the Yeast Saccharomyces Cerevisiae: Life Cycle and Inheritance*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 289–339.
4. White, J. H., Lusnak, K. & Fogel, S. (1985) *Nature (London)* **315**, 350–352.
5. Nicolas, A., Treco, D., Schultes, N. P. & Szostak, J. W. (1989) *Nature (London)* **338**, 35–39.
6. Nag, D. K., White, M. A. & Petes, T. D. (1989) *Nature (London)* **340**, 318–320.
7. Holliday, R. (1964) *Genet. Res.* **5**, 282–304.
8. Radding, C. M. (1982) *Annu. Rev. Genet.* **16**, 405–437.
9. Bishop, D. K., Andersen, J. & Kolodner, R. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3713–3717.
10. Kramer, B., Kramer, W., Williamson, M. S. & Fogel, S. (1989) *Mol. Cell. Biol.* **9**, 4432–4440.
11. Williamson, M. S., Game, J. C. & Fogel, S. (1985) *Genetics* **110**, 609–646.
12. Bishop, D. K., Williamson, M. S., Fogel, S. & Kolodner, R. D. (1987) *Nature (London)* **328**, 362–364.
13. Resnick, M. A. (1976) *J. Theor. Biol.* **59**, 97–106.
14. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. (1983) *Cell* **33**, 25–35.
15. Rose, M., Grisafi, P. & Botstein, D. (1984) *Gene* **29**, 110–124.
16. Tschumper, G. & Carbon, J. (1980) *Gene* **10**, 157–166.
17. Kramer, W., Kramer, B., Williamson, M. S. & Fogel, S. (1989) *J. Bacteriol.* **171**, 5339–5346.
18. Beacham, I. R., Schweitzer, B. W., Warrick, H. M. & Carbon, J. (1984) *Gene* **29**, 271–279.
19. Levinson, A., Silver, D. & Seed, B. (1984) *J. Mol. Appl. Genet.* **2**, 507–517.
20. Sherman, F., Fink, G. R. & Hicks, J. B. (1982) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Fischer, S. G. & Lerman, L. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1579–1583.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Myers, R. M., Lumelsky, N., Lerman, L. S. & Maniatis, T. (1985) *Nature (London)* **313**, 495–498.
24. Feinberg, A. P. & Vogelstein, B. A. (1983) *Anal. Biochem.* **132**, 6–13.
25. Fox, M. S. (1978) *Annu. Rev. Genet.* **12**, 47–68.
26. Sun, H., Treco, D., Schultes, N. P. & Szostak, J. W. (1989) *Nature (London)* **338**, 87–90.
27. Cao, L., Alani, E. & Kleckner, N. (1990) *Cell* **61**, 1089–1101.
28. White, C. I. & Haber, J. E. (1990) *EMBO J.* **9**, 663–673.
29. Nag, D. K. & Petes, T. D. (1990) *Genetics* **125**, 753–761.