Recombination Events in *Neurospora crassa* May Cross a Translocation Breakpoint by a Template-Switching Mechanism

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ABSTRACT

To assist investigation of the effect of sequence heterology on recombination in *Neurospora crassa*, we inserted the *Herpes simplex thymidine kinase* gene (*TK*) as an unselected marker on linkage group I, giving a gene order of *Cen–his-3–TK–cog–lpl*. We show here that in crosses heterozygous for *TK*, conversion of a *his-3* allele on one homolog is accompanied by transfer of the heterologous sequence between *cog* and *his-3* from the other homolog, indicating that recombination is initiated centromere-distal of *TK*. We have identified a 10-nucleotide motif in the *cog* region that, although unlikely to be sufficient for hotspot activity, is required for high-frequency recombination and, because conversion of silent sequence markers declines on either side, may be the recombination initiation site. Additionally, we have mapped conversion tracts in His^+ progeny of a translocation heterozygote, in which the translocation breakpoint separates *cog* from the 5′ end of *his-3*. We present molecular evidence of recombination on both sides of the breakpoint. Because recombination is initiated close to *cog* and the event must therefore cross the translocation breakpoint, we suggest that template switching occurs in some recombination events, with repair synthesis alternating between use of the homolog and the initiating chromatid as template.

MEIOTIC recombination is not initiated at random along eukaryote chromosomes but occurs more frequently close to sites known as recombination hotspots (Holliday 1968). Hotspots have been found in many eukaryotes, including mammals (Steinmetz *et al.* 1986; Thomsen *et al.* 1989; Shiroishi *et al.* 1993), plants (Brown and Sunderesan 1991; Patterson *et al.* 1995), *Saccharomyces cerevisiae* (Nicolas *et al.* 1989; Schultes and Szostak 1990; Symington *et al.* 1991; Detloff *et al.* 1992; Malone *et al.* 1992), and other fungi (Gutz 1971; Stamberg and Koltin 1973; MacDonald and Whitehouse 1979; Rossignol *et al.* 1988) including *Neurospora crassa* (Angel *et al.* 1970; Bowring and Catcheside 1998; Yeadon and Catcheside 1998).

Although we have known for more than 30 years that the Neurospora hotspot *cog* is a recombination initiator, the position of the initiating event was uncertain. *cog* is naturally polymorphic among laboratory strains, with alleles that differentially influence recombination in the *his-3* region. Crosses including the dominant allele cog^L show a 6-fold increase in allelic recombination in *his-3* and four times the frequency of crossing over in the *his-3* to *ad-3* interval when compared to crosses homozygous for cog^E (Angel *et al.* 1970; Yeadon and Catcheside 1995). Activity of both *cog* alleles is regulated by the polymorphic unlinked *trans*-acting gene *rec-2*. The dominant allele, $rec\text{-}2^+$, reduces recombination at *his-3* up to 30-fold (Angel *et al.* 1970), to the same low level regardless of the *cog* alleles present in a cross. *cog* was mapped to linkage group I between the flanking markers *his-3* and *ad-3* (Angel *et al.* 1970) and has been shown to be centromeredistal of the 3′ end of the *his-3* coding sequence (Bowring and Catcheside 1991) within a region 2.3–3.2 kb from the 3′ end of *his-3* (Yeadon and Catcheside 1995) and adjacent to the 5′ end of the *lyso*phospho*l*ipase (*lpl*) gene (Yeadon and Catcheside 1999).

Mapping of conversion tracts in prototrophic progeny of crosses heteroallelic for *his-3* mutants revealed an unselected peak of conversion frequency 3.2 kb distal of *his-3*, suggesting that recombination is initiated in this region (Yeadon and Catcheside 1998). Studies of recombination in a translocation heterozygote (Catcheside and Angel 1974) showed that the breakpoint of the reciprocal translocation *his-3* TM429 T(I;VII) separates the recombination initiation site from the centromere-proximal end of *his-3*. Despite this separation, crosses heterozygous for TM429 and proximal *his-3* point mutations (thus separated from *cog* by the breakpoint) yield a high frequency of prototrophic recombinants, provided that cog^L is on the normal-sequence chromosome. Catcheside and Angel (1974) concluded that cog^L can stimulate the frequency of recombination events *in cis* across the translocation breakpoint.

Together, the nature of the TM429 recombination data and the known position of *cog* make it appear likely that recombination is initiated at *cog*. If so, this raises the question of how recombination spans a region in which

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translocation and normal-sequence chromosomes are paired. Catcheside (1986) suggested *cog* might act by stimulating breaks in an extended region of the chromosome surrounding the hotspot, allowing initiation to occur on the other side of the translocation breakpoint. However, the data presented in this article indicate that recombination is initiated at *cog* and that recombination events must cross the translocation breakpoint.

MATERIALS AND METHODS

Culture methods and media: These were as described previously (Bowring and Catcheside 1996), except that crosses were supplemented with 200 μg/ml L-histidine, 500 μg/ml L-alanine, 200 μg/ml adenine, 500 μg/ml L-arginine, and 400 μg/ml L-lysine as required. Vegetative cultures were supplemented with 200 μg/ml L-histidine, 500 μg/ml L-alanine, 400 μg/ml adenosine, 500 μg/ml L-arginine, and 400 μg/ml L-lysine as required.

Molecular methods: Restriction enzymes, Klenow, and T4 DNA ligase were supplied by New England Biolabs (Beverly, MA) and were used according to the manufacturer's instructions. The *Hin*dIII/*Eco*RI fragment of pNK2 (Sachs *et al.* 1997) was extracted from agarose using the JETsorb gel extraction kit supplied by Genomed. The polymerase used for PCR was RedHot supplied by Abgene. PCR amplification used a Perkin-Elmer (Norwalk, CT) 2400 thermal cycler. Cycling conditions included 1.8 mM $MgCl_2$, an annealing temperature of 52°, and 30 cycles. PCR products were passed through Ultra Clean PCR purification columns (MoBio) prior to sequencing. Sequencing was provided by the Australian Genome Research Facility.

Construction of plasmid vectors and strains: We constructed two vectors, pDV2 and pDV3 (Figure 1), each containing the sequence from 432 to 2644 of pNEB193, *his-3* of *N. crassa* 5′-truncated at +332 (to prevent ectopic insertions giving rise to a His^+ phenotype), cog^L and *lpl* 3′-truncated at +1489. pDV2 includes the *his-3* mutation K26, a T to C transition at +1502 leading to replacement of phenylalanine by serine at codon 501, and pDV3 includes the *his-3* mutation K480, an A to T transversion at +2606 leading to replacement of lysine by methionine at codon 848. As both K26 and K480 inactivate histidinol dehydrogenase (Catcheside and Angel 1974), Neurospora strains with these mutations require histidine for growth. In each vector, 1.75 kb of noncoding sequence between *cog* and *his-3* is deleted and replaced by a multiple cloning site providing *Xba*I, *Pme*I, *Mlu*I, *Nde*I, and *Spe*I sites for insertion of exogenous DNA (Figure 1).

IgGκB, a human immunoglobulin kappa light chain gene, was cloned into *Xba*I/*Spe*I of pDV2 and pDV3 (Figure 1), generating pDV2-B5 and pDV3-B5, respectively. pNK2 (Sachs *et al.* 1997) was digested with *Hin*dIII and *Eco*RI, giving a 2-kb fragment that includes *TK* inserted between the Neurospora *arg-2* promoter and terminator sequences (Sachs *et al.* 1997). The *arg2-TK* fragment, after end-filling with Klenow, was ligated into the *Pme*I site of pDV2 and pDV3 (Figure 1), giving pJY64 and pJY65, respectively.

We also constructed recipient Neurospora strains T11644, *A* mating type, and T11630 and T11631, *a* mating type. Each of the three strains carries the K458 allele of *his-3*, cog^L, and *rec-2*, to allow high-frequency initiation of recombination at *cog*. K458 is a G to A transition at +1018 in codon 339 leading to the replacement of glutamic acid by lysine and inactivation of phosphoribosyl-ATP pyrophosphohydrolase. As both K26 and K480 mutations inactivate the same enzymatic function of the *his-3* protein, strains carrying these alleles do not complement one another, but each complements K458 (Catcheside and Angel 1974). Thus heterokaryons carrying K458 and either K26 or K480 will grow without histidine. As Neurospora spheroplasts are multinucleate, transplacement (Figure 2) of a pDV-derived construct into one nucleus results in a heterokaryon that carries both K26 (or K480) and K458 *his-3* alleles and is therefore able to grow without histidine supplementation.

Plasmid constructs were linearized by restriction with one of the four enzymes with a single site in the vector DNA (*Ahd*I, *Pvu*I, *Pvu*II, and *Sph*I; Figure 1) and transformed (Bowring and Catcheside 1993) into spheroplasts (Case *et al.* 1979)

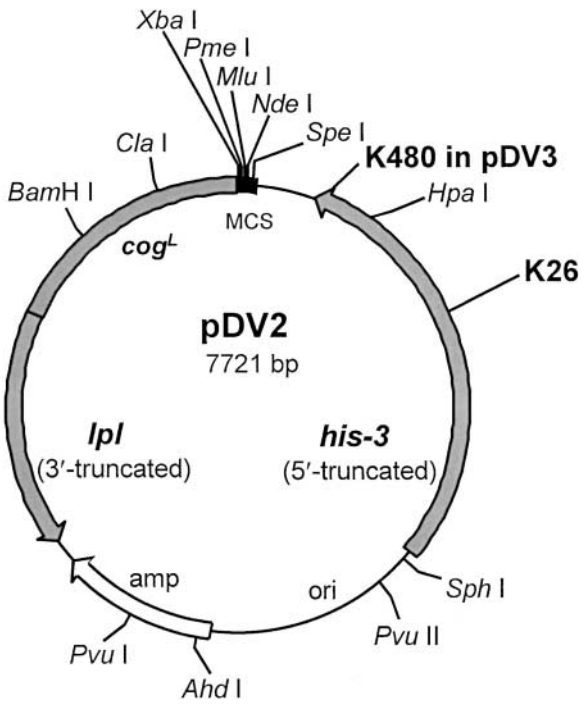

Figure 1.—Vectors pDV2 and pDV3. Each vector contains a 5′-truncated *his-3* gene, cog^L and *lpl*, 3′-truncated. A sequence of 1.75 kb between *his-3* and *cog* has been replaced by a multiple cloning site. With these vectors, sequences cloned into the multiple cloning site can be inserted in chromosome I in place of the noncoding sequences between *his-3* and *cog*. pDV2 includes the *his-3* point mutation K26 and pDV3 the *his-3* point mutation K480.

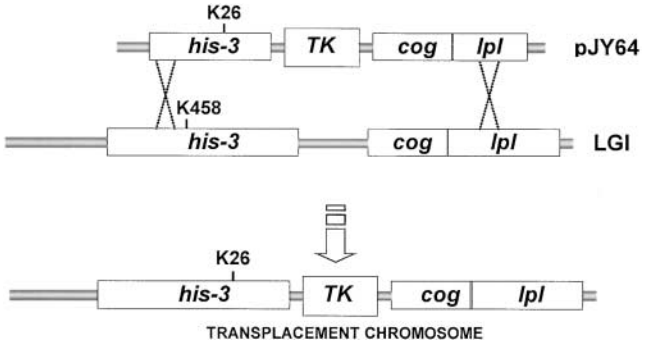

Figure 2.—Transplacement of exogenous DNA cloned in pDV into chromosome I. The exogenous DNA replaces noncoding sequences between *his-3* and *cog*. The centromere is to the left side of the figure.

TABLE 1

Neurospora stocks

Stock no.	Genotype
T2326	*a; fl*
T2327	*A; fl*
T4394	*a, his-3* K504, cog^E, *ad-3; rec-2*
T11249	*arg-1, A; am, rec-2*
T11250	*arg-1, A; rec-2*
T11255	*arg-1, A; am, rec-2*
T11257	*arg-1, A; rec-2*
T11264	*arg-1, A; am, rec-2*
T11281	*A, his-3* K1201, cog^L, *ad-3; am, rec-2*
T11318	*a, his-3* K26, cog^E*; am, rec-2*
T11439	*arg-1, a, his-3* TM429, cog^L*; rec-2*
T11440	*arg-1, a, his-3* TM429, cog^L*; rec-2*
T11442	*arg-1, a, his-3* TM429, cog^L*; rec-2*
T11630	*a, his-3* K458, cog^L*; rec-2*
T11631	*a, his-3* K458, cog^L*; rec-2*
T11644	*A, his-3* K458, cog^L*; rec-2*
T11668	*a, lys-4,* cog^E*, ad-3; am, rec-2*
T11670	*a, lys-4,* cog^E, ad-3*; rec-2*$^+$
T11681	*a, lys-4, his-3* K480, cog^E*, ad-3; am, rec-2*
T11686	*A, his-3* K26, (*arg2-TK*), cog^L*; rec-2*
T11690	*A, his-3* K480, (*arg2-TK*), cog^L*; rec-2*
T11704	*A, his-3* K480, (*arg2-TK*), cog^L*; rec-2*
T11725	*A, his-3* K26, (*arg2-TK*), cog^L*; rec-2*
T11726	*a, his-3* K26, (*arg2-TK*), cog^L*; rec-2*
T11730	*A, his-3* K26, (*arg2-TK*), cog^L*; rec-2*
T11738	*A, his-3* K480, (IGκB), cog^L*; rec-2*
T11739	*a, his-3* K26, (IGκB), cog^L*; rec-2*
T11761	*a, his-3* K480, cog^L*; am, rec-2*
T11762	*a, his-3* K480, cog^L*; am, rec-2*
T12002	*A, his-3* K480, *egl3,* cog^L*; rec-2*
T12004	*a, his-3* K26, *egl3,* cog^L*; rec-2*

The *arg-1* allele is K166, *am* is K314, *ad-3* is K118, and *lys-4* is STL4. All strains except T2326 and T2327 include the colonial temperature-sensitive mutation *cot-1* C102t.

made from T11644, T11630, or T11631. The transformation mix was plated onto minimal medium to select heterokaryotic colonies, each of which grows from a spheroplast with a nucleus carrying a transplacement chromosome (Figure 2). Monokaryons were obtained by plating microconidia (Ebbole and Sachs 1990) on histidine-supplemented medium. The *his-3* allele in each of the resulting histidine-requiring monokaryotic cultures was identified by complementation of the original *his-3* K458 recipient strain (T11644, T11630, or T11631). In cultures that formed histidine-independent heterokaryons when mixed with conidia of the recipient strain, the *his-3* K458 allele in the untransformed strain has been replaced by the K26 or K480 allele provided by the transforming DNA.

T11738 and T11739 (Table 1) were made by transfection of T11644 with pDV3-B5 and T11631 with pDV2-B5, respectively. PCR was used to identify presence of the IgGκB sequence in each transformed strain, followed by Southern analysis to determine correct placement and complete insertion of the construct (data not shown).

The putative TK^+ strains T11686 and T11690 (Table 1) were made by transfection of T11630 with pJY64 and pJY65, respectively. Prior to Southern analysis (data not shown), the presence of the *arg2-TK* construct was determined by sensitivity to fluorodeoxyuridine (FdU). Untransformed strains are highly resistant to FdU due to the lack of native thymidine kinase activity in Neurospora (Sachs *et al.* 1997). Conidia were inoculated onto solid medium (5 μM FdU, 2% Vogels salts, 2% Difco agar, 2% sucrose, and supplements as required) and incubated at 34° for 2–3 days.

Since T11686 and T11690 are both the same mating type, *his-3* progeny of both mating types were extracted from crosses of each transformed strain to *lys-4 his-3*$^+$ *ad-3* strains T11668 and T11670 (Table 1) and tested for sensitivity to FdU. T11704 was extracted from a cross between T11670 and T11690. T11725 and T11726 were extracted from a cross between T11670 and T11686. T11730 was extracted from a cross between T11668 and T11686.

T12002 and T12004 (Table 1), generated by transfection of T11644 and T11630, respectively, with pDV-derived constructs each including an endogluconase gene from a different fungal species, were supplied by Neugenesis (San Carlos, CA). T11249–T11264 (Table 1) are histidine prototrophs isolated for conversion tract mapping (Yeadon and Catcheside 1998), in each of which the *cog* region is mosaic and made up of sequences derived from both cog^E and cog^L.

Extraction and testing of recombinant progeny: Spores from crosses heteroallelic for *his-3* (K26/K480) and heterozygous for *TK*, with the *his-3* alleles in a variety of configurations with respect to *TK* and *cog* alleles (Figure 3), were plated onto medium (2% Vogels salts, 2% Difco agar, 4% sorbose, 0.1% glucose, 0.1% fructose, and supplements as required) lacking histidine and incubated at 25° for 20 hr and then at 34° for 24 hr. Colonies were picked into slopes (2% Vogels salts, 2% agar, 2% sucrose, and supplements as required) and incubated at 25° for 5 days. Each was then tested for FdU sensitivity to determine presence or absence of *TK*. A total of 32 His$^+$ progeny were tested from each cross of type A (T11704 × T11739; T11704 × T12004) and B (T11738 × T11726; T12002 × T11726; T11761 × T11730; T11760 × T11730) and 64 from each cross of type C (T11704 × T11318) and D (T11681 × T11725; Figure 3).

Identification of a short sequence required for the high-frequency recombination phenotype of cog^L: More than 1500 bp of sequence, including the entire 930-bp *cog* region (Yeadon and Catcheside 1995), was PCR amplified and sequenced in each of the strains with mosaic versions of *cog* (T11249, T11250, T11255, T11257, and T11264). Primers used for PCR and sequencing were C1F (TCG ACG AAG AAA ATA CGC G), C1R (CCG CTA CTC CAC ACA CCC), C2F (GTA GAG GCG CTT GGA AGT), C2R (TCG CCC AAG AAA AAA AGA GA), C3F (CAC TTC CCT TGG TCT GGG), C3R (CAT CGC GTT TGG GGT ATC), C9F (GCA ACT TGC CGT TCG AGC), and C9R (TCG GTT ATC CGC AGC AGC). Primer pairs used for PCR were C1F/C2R, C2F/C3R, and C3F/C9R. Sequencing of each PCR product used all primers (Yeadon and Catcheside 1998) within the product. A contiguous sequence was generated for each strain using the program Sequencher (v 4.0.5, supplied by Gene Codes, Ann Arbor, MI) to edit and align the individual sequences. Each contiguous mosaic *cog* region sequence was aligned with cog^L and cog^E sequences from ST74A and Lindegren Y8743 strains, respectively (Yeadon and Catcheside 1995) using CLUSTALW, one of the suite of alignment programs available from the Australian National Genome Information Service. At each of the 13 variant positions within the *cog* region (Yeadon and Catcheside 1995), the parental origin (cog^L or cog^E) of the base was determined.

The *cog* phenotype of a strain can be determined by assessing the frequency of either interallelic recombination in *his-3* or intergenic (*his-3* to *ad-3*) recombination (Angel *et al.* 1970). Since the strains with mosaic *cog* region sequences are

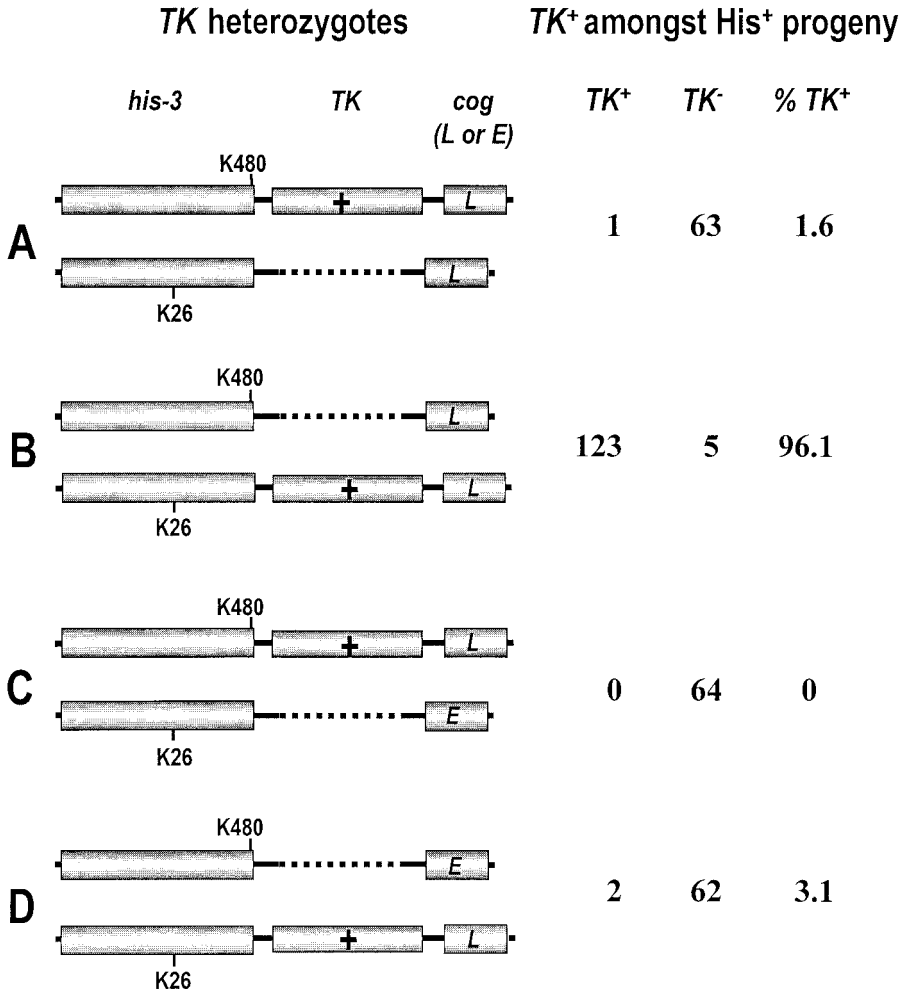

FIGURE 3.—The frequency of TK^+ among His^+ progeny of crosses heterozygous for *TK*. (A) Homozygous cog^L, K480, and *TK in cis* (T11704 × T11739 and T11704 × T12004). (B) Homozygous cog^L, K480, and *TK in trans* (T11738 × T11726, T12002 × T11726, T11761 × T11730, and T11760 × T11730). (C) Heterozygous cog^L with K480, *TK*, and cog^L *in cis* (T11704 × T11318). (D) Heterozygous cog^L with K26, *TK*, and cog^L *in cis* (T11681 × T11725). A dashed line indicates that a sequence other than *TK* (IGκB, *egl3*, or the native sequence) is present in the *his-3–cog* interval. The centromere is to the left side of the figure.

all his^+ ad^+, the *cog* phenotypes were determined by assessing the frequency of intergenic recombination. Each was crossed to a *his-3 ad-3* cog^E *rec-2* tester strain (T4394; Table 1) and the *his-3* and *ad-3* genotypes of 256 unselected progeny of each cross were ascertained by determining the requirement for histidine and adenosine, respectively. The frequency of exchange in the *his-3* to *ad-3* interval was determined by calculating the percentage of recombinant ($his\text{-}3^+$ *ad-3* and *his-3* $ad\text{-}3^+$) progeny.

Extraction and conversion tract analysis of His^+ progeny of a translocation heterozygote: Spores from crosses (T11281 crossed to T11439, T11440, and T11442; Table 1) heterozygous for the *his-3* translocation mutant TM429 and homozygous cog^L and *rec-2* (Figure 4) were plated onto medium lacking histidine as described above. For 11 His^+ progeny from the cross to T11439, 10 from that to T11440, and 7 from that to T11442, the *ad-3* and *arg-1* genotypes were ascertained by determining the requirement for adenosine and arginine, respectively. Mating type was determined by crosses to the mating-type tester strains T2326 and T2327 (Table 1). The parental origin of sections of sequence between the 5′ end of *his-3* and the proximal end of the *cog* region was determined in each of the His^+ progeny by detection of restriction site polymorphism in PCR products, as described previously (YEADON and CATCHESIDE 1998).

RESULTS

Comparison of mosaic *cog* region sequences and determination of *cog* phenotypes: Of the five His^+ recombi-

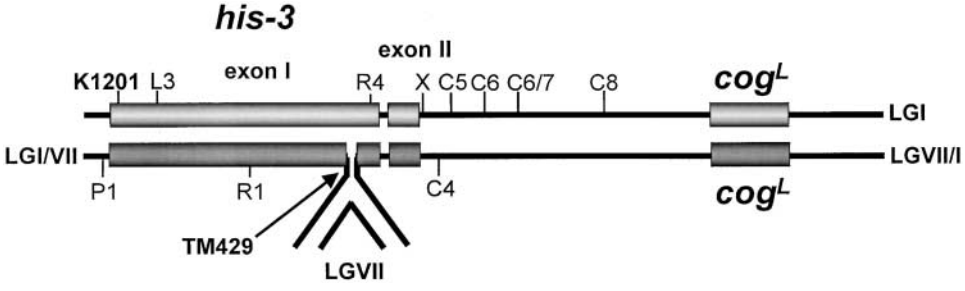

FIGURE 4.—A diploid heterozygous for the reciprocal translocation *his-3* TM429 T(I;VII) and K1201, a *his-3* point mutation proximal to the breakpoint. Polymorphic sites used to map conversion tracts are indicated by a vertical line. The centromere is to the left side of the figure.

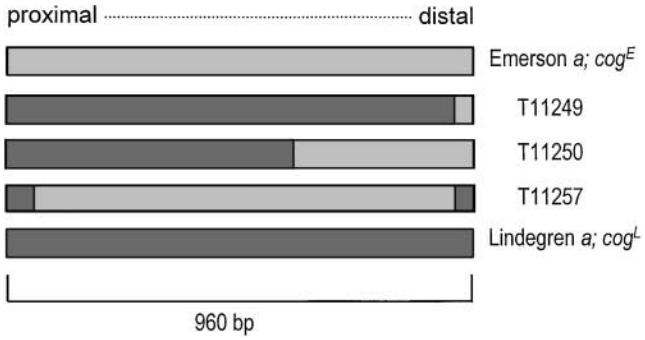

FIGURE 5.—*cog* region sequences in strains with mosaic versions of *cog*. The centromere and *his-3* are to the left side of the figure.

nant strains with mosaic versions of *cog* (YEADON and CATCHESIDE 1998), T11250, T11255, and T11264 were found to have identical sequences in the 930-bp *cog* region (YEADON and CATCHESIDE 1995). Therefore, the *cog* phenotype was determined only for T11250, T11249, and T11257, each of which has a different mosaic of cog^L and cog^E sequences (Figure 5). In crosses homozygous for the recessive *rec-2* allele, recombination in the *his-3* to *ad-3* interval is 5–10% in the presence of cog^L and 1–3% in its absence (ANGEL *et al.* 1970; CATCHESIDE and ANGEL 1974). The frequency of exchange in the *his-3* to *ad-3* interval in progeny of T11257 crossed to the cog^E tester strain T4394 is significantly higher (6.64%) than in those of T11249 (1.17%) and T11250 (1.95%; Table 2; $\chi^2 = 13.97$, $P < 0.001$), suggesting that, while T11249 and T11250 are cog^E, T11257 is cog^L in phenotype.

Although the *cog* region sequences in T11249 and T11250 are mostly of cog^L (Lindegren 25*a*) origin, the centromere-distal end of each sequence is from cog^E (Emerson *a*; Figure 5). In contrast, T11257 has sequence from cog^L only at the proximal and distal ends of the *cog* region (Figure 5). Sequence at the distal end of *cog* in T11257 includes two bases that are variant in the naturally occurring *cog* alleles. The 10-base sequence CCCTACGGTT is bounded by these two bases, shown here for cog^L; the underlined C is T and the T is A in cog^E. We can conclude that one or both of these bases is essential for the high-frequency recombination phenotype of cog^L. Of the other 11 sequence variations that distinguish cog^L from cog^E (YEADON and CATCHESIDE 1995), we can conclude that the central 6 (including a 9-base palindrome arising from an insertion of TGG in cog^L) are not relevant to the phenotype of *cog*. It is not yet possible to determine whether the remaining three variations at the proximal end of *cog* (a single-base variation, an insertion of TGGGG, and a poly(T) that is two bases longer in cog^L) are required for the high-frequency recombination phenotype of cog^L. In addition, since cog^E retains weak hotspot activity, even if the two nucleotides identified above are the only positions essential for the high-frequency recombination phenotype of cog^L, other sequences must be necessary for *cog* to function as a recombinator.

Segregation of *TK* in His$^+$ progeny of crosses heteroallelic for K26/K480 and heterozygous for *TK*: All strains successfully transformed with the *arg2-TK* (SACHS *et al.* 1997) constructs pJY64 and pJY65 (T11686 and T11690) and their *his-3* progeny (T11704, T11725, T11726, and T11730) showed little growth in the presence of FdU when compared to the untransformed TK^- strain (T11630), demonstrating the expected FdU sensitivity due to an active thymidine kinase gene.

Of 32 His$^+$ progeny of T11704 × T11739 (*his-3* K480 *TK* cog^L × *his-3* K26 IgGκB cog^L), one was TK^+, and of 32 His$^+$ progeny of T11704 × T12004 (*his-3* K480 *TK* cog^L × *his-3* K26 *egl3* cog^L), none was TK^+ (Fisher's exact test: $P_{exact} = 0.38$). Of 32 His$^+$ progeny of T11738 × T11726 (*his-3* K480 IgGκB cog^L × *his-3* K26 *TK* cog^L), 31 were TK^+, and of 32 His$^+$ progeny of T12002 × T11726 (*his-3* K480 *egl3* cog^L × *his-3* K26 *TK* cog^L), 30 were TK^+ (Fisher's exact test: $P_{exact} = 0.50$). Of 32 His$^+$ progeny of T11761 × T11730 and 32 of T11762 × T11730 (*his-3* K480 cog^L × *his-3* K26 *TK* cog^L), 32 and 30, respectively, were TK^+ (Fisher's exact test: $P_{exact} = 0.25$). The frequency of TK^+ among His$^+$ progeny is thus unaffected by the identity of the sequence in the *his-3–cog* interval; so results were pooled by cross type (Figure 3), reflecting only the arrangement of K26, K480, cog^L, and *TK*.

The only cross type (Figure 3) that yielded a majority (96%) of recombinant His$^+$ progeny showing FdU sensitivity (TK^+) was type B (Figure 3), in which *TK* and K480 are on different chromosomes, both of which carry cog^L. His$^+$ progeny from cross types A and C (Figure 3), in which K480 and *TK* are on the same chromosome, are mostly TK^- (98.4 and 100%, respectively). Data from these three cross types show either that events leading

TABLE 2

Recombination in the *his-3* to *ad-3* interval in crosses of strains with mosaic versions of *cog* to a cog^E tester

Cross	*his*$^+$ *ad*$^+$	*his-3 ad-3*	*his*$^+$ *ad-3*	*his-3 ad*$^+$	Parental	Recombinant	rf (%)
T11249 × T4394	147	104	4	1	251	5	1.95
T11250 × T4394	135	118	2	1	253	3	1.17
T11257 × T4394	116	123	9	8	239	17	6.64

rf (recombination frequency) was calculated by summing the recombinant (*his*$^+$ *ad*$^+$ and *his-3 ad-3*) and dividing by the total number of progeny × 100% (CATCHESIDE 1979).

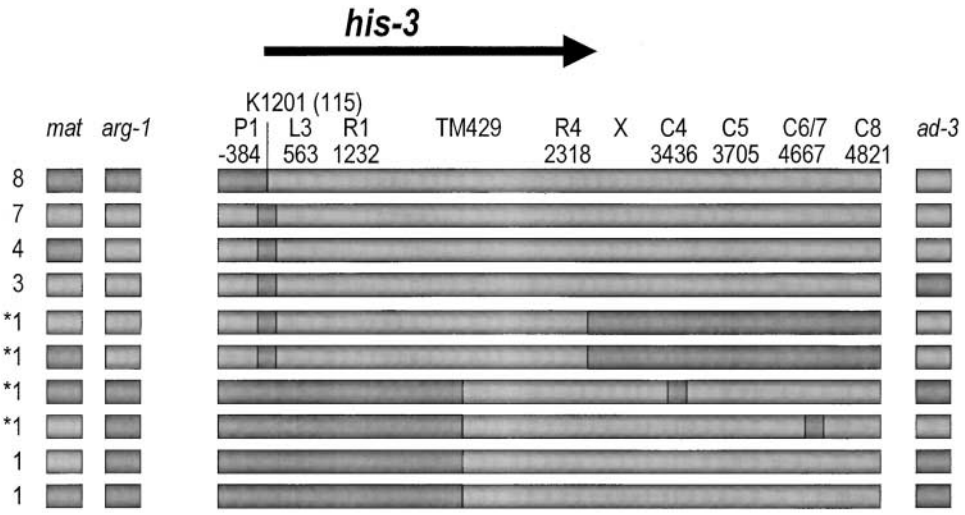

FIGURE 6.—Mapping of conversion tracts in His$^+$ progeny of crosses heterozygous for the TM429 translocation. The darker shading indicates sequences from the normal sequence parent, T11281, and the lighter shading those from the translocation mutant strains (T11439, T11440, and T11442). The location of the TM429 breakpoint is not known exactly, but it falls within the 313 bp (P. J. YEADON and D. E. A. CATCHESIDE, unpublished data) flanked by the R3 PCR primers (YEADON and CATCHESIDE 1998) between the R1 and R4 markers. The centromere lies between *his-3* and *arg-1*; the gaps indicate substantial physical distances.

to conversion of K480, the closer mutant site to *cog*, also transfer the sequence between *cog* and the mutant site to the recipient chromosome, or that most of these prototrophs result from reciprocal recombination (crossover events) between the mutant sites. Crosstype D (Figure 3) is the only type in which K26, on the same chromosome as *TK*, is expected to be converted with high frequency, as it lies on the only chromosome that carries cog^L. For this cross type, a crossover between K26 and K480 would result in His$^+$ progeny that are primarily TK^+. However, since only 3.1% of the His$^+$ progeny are TK^+ (Figure 3), we can conclude that the majority of the prototrophs from crosstype D resulted from conversion of K26 and that the recombination event also transferred the heterologous sequence between *cog* and *his-3* to the recipient chromosome. It is thus likely that most of the His$^+$ progeny from all four cross types result from conversion of the mutant allele on one homolog, accompanied by transfer of the heterologous sequence between *cog* and *his-3* from the other homolog. We therefore conclude that the initiation site for recombination is to the right of *TK* (Figure 3), probably within the *cog* region.

Conversion tracts in His$^+$ progeny of a translocation heterozygote: Of the 28 His$^+$ progeny of the K1201/TM429 translocation heterozygote (Figure 4), 8 (top bar in Figure 6) appear to result from a simple crossover between K1201 and TM429. Of the remaining 20 His$^+$ progeny analyzed, 4 (14% of the total) marked with an asterisk (Figure 6) show evidence of recombination on both sides of the translocation breakpoint.

DISCUSSION

In *S. cerevisiae*, *Schizosaccharomyces pombe*, *Ascobolus immersus*, and *N. crassa*, the closer a site is to a recombination hotspot, the more frequently it is converted, a phenomenon known as polarity (reviewed in NICOLAS and PETES 1994). Thus it is expected that, in His$^+$ progeny from a cross heteroallelic for *his-3*, homozygous for *rec-2* (to maximize recombination initiated at *cog*), and homozygous for cog^L (to ensure that recombination is initiated with equal frequency on each chromosome), the chromosome carrying the mutant site closer to *cog* (K480) would usually be the recipient of information if the other chromosome carries the more distant mutant site K26 (Figure 3). In *N. crassa*, *S. cerevisiae*, and *S. pombe*, if the cross is heterozygous for a hotspot, the chromosome carrying the more active hotspot is usually the recipient of information and the mutant site *in cis* to the active hotspot is the more frequently converted to wild type (ANGEL *et al.* 1970; GUTZ 1971; CATCHESIDE 1977; NICOLAS *et al* 1989; YEADON and CATCHESIDE 1998). As predicted, our data show that the chromosome bearing K480 is usually the recipient of information (Figure 3, A–C) unless the only copy of cog^L is on the same chromosome as K26 (Figure 3D). In addition, however, in crosses heterozygous for *TK*, the chromosome experiencing conversion of the mutant site to wild type is also the recipient of the heterologous sequence between *cog* and *his-3* from the other homolog (Figure 3). This could happen only if the initiating event for recombination occurs on the *cog* side of the *TK* insertion site. Thus initiation must occur either within the *cog* region (YEADON and CATCHESIDE 1995) or in the 330 bp between *TK* and *cog*.

In yeast, where recombination is initiated by double-strand breaks (DSB; ORR-WEAVER and SZOSTAK 1983; SUN *et al.* 1989; CAO *et al.* 1990; FAN *et al.* 1995), hotspots are usually close to the 5′ end of genes (WU and LICHTEN 1994). We have shown here (Figure 5 and Table 2) that a 10-nucleotide motif, 16 bp from the *lpl* CAAAT sequence and 140 bp 5′ of the presumptive *lpl* start codon (YEADON and CATCHESIDE 1999), is required for

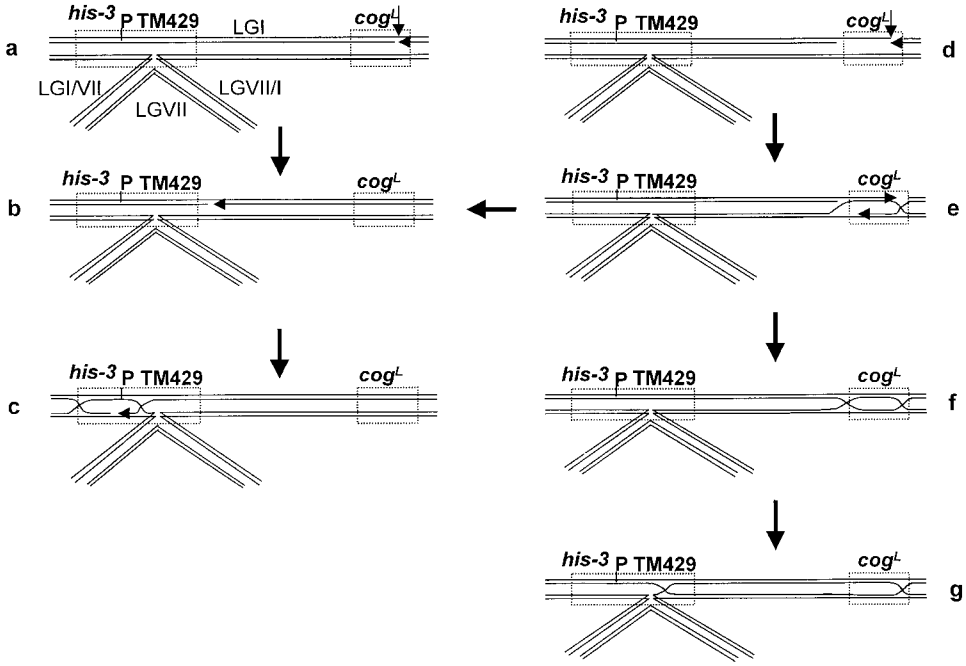

FIGURE 7.—Recombination models and the TM429 translocation breakpoint. (1) The model of CATCHESIDE and ANGEL (1974): Recombination is initiated by a nick in a single strand at *cog* (a). DNA synthesis can copy from either the homolog or the initiating chromatid. Copying from the initiating chromatid causes further degradation of the 5′ end, leading to a traveling nick (b). After the nick passes the breakpoint (c), a recombination intermediate resulting from copying from the translocation chromosome can yield a His$^+$ chromatid. (2) The double-strand break-repair model cannot explain how recombination crosses the breakpoint. Recombination is initiated by a DSB at *cog* and the 5′ ends of the break are resected to give 3′ overhanging ends (d). Each of the 3′ ends can invade the homolog, displacing the strand of like polarity, although only one such invasion is shown in the figure (e). DNA synthesis proceeds to fill the gap (e). Ligation of ends results in Holliday junctions (f). Although the junctions can migrate in either direction, they cannot cross the breakpoint (g). (3) The template-switching model: Recombination is initiated by a DSB at *cog* (d and e). Ligation of ends does not occur at this early stage, if at all, so the recombination intermediate can unravel. DNA synthesis can switch readily between the homolog and the initiating chromatid (or the sister, not shown in this figure), provided that the sequences of the homologs are sufficiently similar for binding of the end to occur. Thereafter, recombination proceeds as described for the CATCHESIDE and ANGEL (1974) model (b and c), except that, since there are two 3′ ends, the event may be bidirectional (only events proximal of *cog* are shown in this figure). A His$^+$ chromatid from a recombination intermediate formed after the breakpoint is crossed may have patches of sequence from both parents in the region between *cog* and the TM429 breakpoint. The centromere is to the left of the figure and *lpl* is to the right. TM429 is a reciprocal translocation within the *his-3* coding sequence, such that the 5′ end of *his-3* is joined to linkage group VII (LGI/VII). The other half of LGVII is joined to the 3′ end of *his-3* (LGVII/I). P is a point mutation proximal to TM429 and present in the normal-sequence chromosome.

the high-frequency recombination phenotype of *cog^L*. In His$^+$ progeny of a *his-3* heterozygote, conversion of silent sequence markers peaks at the centromere-distal end of the *cog* region (YEADON and CATCHESIDE 1998), close to or coincidental with the motif necessary for the *cog^L* phenotype. We therefore consider it likely that recombination initiated by *cog* commences within the *cog* region (YEADON and CATCHESIDE 1995), probably close to the 5′ end of *lpl* and possibly at the 10-nucleotide motif.

Recombination events initiated by *cog^L* can pass the TM429 translocation breakpoint (CATCHESIDE and ANGEL 1974) to give His$^+$ recombinants in diploids with point mutations beyond the breakpoint, provided that *cog^L* is on the normal-sequence chromosome. CATCHESIDE and ANGEL (1974) proposed a single-strand break at *cog* as the initiating event (Figure 7a). The 3′ end of the nick then acts as a primer for DNA synthesis, using the complementary strand of the same duplex as template (Figure 7b). After crossing the translocation breakpoint, the 3′ end of the traveling nick could initiate an exchange and generate a His$^+$ recombinant spore (Figure 7c). The later demonstration that recombination in yeast is initiated by a double-strand break (ORR-WEAVER and SZOSTAK 1983) promoted consideration of how recombination events, if initiated by a DSB in Neurospora (Figure 7d), might pass the TM429 breakpoint.

Because repair of a DSB (Figure 7d) involves DNA synthesis using the homologous duplex as template (Figure 7e) and Holliday junctions formed distal of the TM429 interchange cannot pass the breakpoint to give conversion of proximal *his-3* alleles (Figure 7, f and g), CATCHESIDE (1986) suggested that *cog* might act by stimulating strand breaks at a distance. The suggested mechanism is that *cog* is analogous to the recognition site of a Type I restriction endonuclease, which cuts some distance from the specificity site, thus allowing initiation to occur beyond the TM429 breakpoint (CATCHESIDE 1986).

Our demonstration that exchange events are predominantly initiated close to *cog*, at least in diploids with extensive sequence heterology between *cog* and *his-3*, argues that repair synthesis is initiated at or close to *cog* rather than at any substantial distance (Figure 7d). In that case, in a TM429 heterozygote, repair initiated on the normal-sequence chromosome would involve copying sequence close to *cog* from the chromosome carrying

the translocation (Figure 7e). Since point mutations located beyond the translocation breakpoint experience conversion, sequence copying must return to the initiating chromosome or its sister (Figure 7b) to pass the chromosomal heterology and finally invade and copy from the TM429 chromosome copy once more (Figure 7c). A specific prediction of this hypothesis is that there should be patches of conversion both sides of the breakpoint in some His^+ progeny of crosses heterozygous for TM429 and a *his-3* point mutation. Indeed, this prediction is realized in ~14% of His^+ chromatids from such crosses (Figure 6).

An alternate explanation for the observation of conversion both sides of the breakpoint is that the conversion events on the proximal side of the breakpoint that generate His^+ progeny are initiated 5′ of *his-3* and that 14% of such progeny experience additional recombination events initiated at *cog*. However, in crosses of TM429 to a proximal allele (K504) on a normal-sequence chromosome that carries cog^E, interallelic recombination occurs with a frequency of $11/10^5$ viable spores whether $rec\text{-}2^+$ is present in the cross or not (Catcheside and Angel 1974), showing that the recombination initiation site at the 5′ end of *his-3* is insensitive to $rec\text{-}2^+$. If TM429 is replaced by a base substitution mutation in a similar position to the translocation (K874; Yeadon and Catcheside 1999) on a chromosome that carries cog^L, the presence of $rec\text{-}2^+$ reduces the frequency of His^+ progeny of a cross to K504 from $167.0/10^5$ to $5.1/10^5$ viable spores (Catcheside and Angel 1974), suggesting that initiation at the proximal "hotspot" occurs 20- to 50-fold less frequently than it does at cog^L. In contrast, in crosses of TM429 to another proximal allele (TM428) on a normal sequence chromosome that carries cog^L, $rec\text{-}2^+$ reduces interallelic recombination ~20-fold (Catcheside and Angel 1974), showing the expected effect of the regulatory gene on recombination initiated at cog^L, despite the presence of the translocation. Substitution of K874 for TM429 increases the frequency of His^+ progeny <3-fold, from $18.8/10^5$ (Catcheside and Angel 1974) to $52.7/10^5$ viable spores (Angel *et al.* 1970), suggesting that the translocation has only a small effect on recombination compared to the effect of $rec\text{-}2^+$.

Thus it seems unlikely that the initiation site at the 5′ end of *his-3* could be responsible for the majority of recombination in TM429 heterozygotes, unless recombination at cog^L stimulates events at this site. If this were the case, it is difficult to explain why this stimulation occurs only *in cis* to cog^L without concluding that the "stimulating effect" is due to a single recombination event, initiated at cog^L and crossing the translocation breakpoint.

We therefore suggest that the hypothesis of template switching described above (Figure 7, b–e) is the most likely explanation of our observation (Figure 6) of conversion both sides of the TM429 translocation breakpoint. The template-switching hypothesis also suggests that interruptions to conversion tracts observed in His^+ chromatids from crosses homozygous for normal sequence chromosomes (Yeadon and Catcheside 1998) at least in part may be due to multiple switching between chromatids during repair replication and predicts that some tracts may be interrupted even in recombinants from mismatch repair knockout homozygotes.

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LITERATURE CITED

Angel, T., B. Austin and D. G. Catcheside, 1970 Regulation of recombination at the *his-3* locus in *Neurospora crassa*. Aust. J. Biol. Sci. **23:** 1229–1240.

Bowring, F. J., and D. E. A. Catcheside, 1991 The initiation site for recombination *cog* is at the 3′ end of the *his-3* gene in *Neurospora crassa*. Mol. Gen. Genet. **229:** 273–277.

Bowring, F. J., and D. E. A. Catcheside, 1993 The effect of *rec-2* on repeat-induced point mutation (RIP) and recombination events that excise DNA sequence duplications at the *his-3* locus in *Neurospora crassa*. Curr. Genet. **23:** 496–500.

Bowring, F. J., and D. E. A. Catcheside, 1996 Gene conversion alone accounts for more than 90% of recombination events at the *am* locus of *Neurospora crassa*. Genetics **143:** 129–136.

Bowring, F. J., and D. E. A. Catcheside, 1998 Analysis of conversion tracts associated with recombination events at the *am* locus of *Neurospora crassa*. Curr. Genet. **34:** 43–49.

Brown, J., and V. Sunderesan, 1991 A recombination hotspot in the maize *A1* intragenic region. Theor. Appl. Genet. **81:** 185–188.

Cao, L., E. Alani and N. Kleckner, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:** 1089–1101.

Case, M. E., M. Schweizer, S. R. Kushner and N. H. Giles, 1979 Efficient transformation of *Neurospora crassa* using hybrid plasmid DNA. Proc. Natl. Acad. Sci. USA **76:** 5259–5263.

Catcheside, D. E. A., 1979 Effect of $rec\text{-}2^+$ on the formation of double mutant recombinants in *Neurospora crassa*. Aust. J. Biol. Sci. **32:** 261–265.

Catcheside, D. E. A., 1986 A restriction and modification model for the initiation and control of recombination in *Neurospora*. Genet. Res. **47:** 157–165.

Catcheside, D. G., 1977 *The Genetics of Recombination*. Edward Arnold, London.

Catcheside, D. G., and T. Angel, 1974 A *histidine-3* mutant, in *Neurospora crassa*, due to an interchange. Aust. J. Biol. Sci. **27:** 219–229.

Detloff, P., M. A. White and T. D. Petes, 1992 Analysis of a gene conversion gradient at the *HIS4* locus in *Saccharomyces cerevisiae*. Genetics **132:** 113–123.

Ebbole, D., and M. S. Sachs, 1990 A rapid method for isolation of *Neurospora crassa* homokaryons using microconidia. Fungal Genet. Newsl. **37:** 17–18.

Fan, Q., F. Xu and T. Petes, 1995 Meiosis-specific double-strand breaks at the *HIS4* recombination hotspot in the yeast *Saccharomyces cerevisiae*: control in cis and trans. Mol. Cell. Biol. **15:** 1679–1688.

Gutz, H., 1971 Site specific induction of gene conversion in *Schizosaccharomyces pombe*. Genetics **69:** 317–337.

Holliday, R., 1968 Genetic control of recombination in fungi, pp. 157–174 in *Replication and Recombination of Genetic Material*, edited by W. J. Peacock and R. D. Brock. Australian Academy of Science, Canberra.

MacDonald, M. V., and H. L. K. Whitehouse, 1979 A *buff* spore colour mutant in *Sordaria brevicollis* showing high frequency conversion. Genet. Res. **34:** 87–119.

Malone, R. E., S. A. Bullard, S. Lundquist, S. Kim and T. Tartow-

SKI, 1992 A meiotic gene conversion gradient opposite to the direction of transcription. Nature **359:** 154–155.

NICOLAS, A., and T. D. PETES, 1994 Polarity of meiotic gene conversion in fungi: contrasting views. Experientia **50:** 242–252.

NICOLAS, A., D. TRECO, N. P. SCHULTES and J. W. SZOSTAK, 1989 Identification of an initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. Nature **338:** 35–39.

ORR-WEAVER, T. L., and J.W. SZOSTAK, 1983 Yeast recombination: the association between double-strand gap repair and crossing over. Proc. Natl. Acad. Sci. USA **80:** 4417–4421.

PATTERSON, G. I., K. M. KUBO, T. SHROYER and V. L. CHANDLER, 1995 Sequences required for paramutation of the maize *b* gene map to a region containing the promoter and upstream sequences. Genetics **140:** 1389–1406.

ROSSIGNOL, J.-L., A. NICOLAS, H. HAMZA and A. KALOGEROPOULOS, 1988 Recombination and gene conversion in *Ascobolus*, pp. 23–72 in *The Recombination of Genetic Material*, edited by K. B. LOW. Academic Press, New York.

SACHS, M. S., E. U. SELKER, B. LIN, C. J. ROBERTS, Z. LUO *et al.*, 1997 Expression of herpes virus thymidine kinase in *Neurospora crassa*. Nucleic Acids Res. **25:** 2389–2395.

SCHULTES, N. P., and J. W. SZOSTAK, 1990 Decreasing gradients of gene conversion on both sides of the initiation site for meiotic recombination at the *ARG4* locus in yeast. Genetics **126:** 813–822.

SHIROISHI, T., T. SAGAI and K. MORIWAKI, 1993 Hotspots of meiotic recombination in the mouse major histocompatibility complex. Genetica **88:** 187–196.

STAMBERG, J., and Y. KOLTIN, 1973 Genetic control of recombination in *Schizophyllum commune*: evidence for a new type of regulatory site. Genet. Res. **22:** 101–111.

STEINMETZ, M., D. STEPHAN and K. FISCHER-LINDAHL, 1986 Gene organization and recombinational hotspots in the murine *Major Histocompatibility Complex*. Cell **44:** 895–904.

SUN, H., D. TRECO and J. W. SZOSTAK, 1989 Double strand breaks at an initiation site for meiotic gene conversion. Nature **338:** 87–90.

SYMINGTON, L. S., A. BROWN, S. G. OLIVER, P. GREENWELL and T. D. PETES, 1991 Genetic analysis of a meiotic recombination hotspot on chromosome *III* of *Saccharomyces cerevisiae*. Genetics **128:** 717–727.

THOMSEN, M., M. ABBAL, M. NEUGEBAUER and A. CAMBON-THOMSEN, 1989 Recombination in the *HLA* system. Tissue Antigens **33:** 38–40.

WU, T. C., and M. LICHTEN, 1994 Meiosis-induced double-strand break sites determined by yeast chromatin structure. Science **263:** 515–518.

YEADON, P. J., and D. E. A. CATCHESIDE, 1995 The chromosomal region which includes the recombinator *cog* in *Neurospora crassa* is highly polymorphic. Curr. Genet. **28:** 155–163.

YEADON, P. J., and D. E. A. CATCHESIDE, 1998 Long, interrupted conversion tracts initiated by *cog* in *Neurospora crassa*. Genetics **148:** 113–122.

YEADON, P. J., and D. E. A. CATCHESIDE, 1999 Polymorphism around *cog* extends into adjacent structural genes. Curr. Genet. **35:** 631–637.

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