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

P. Jane Yeadon, J. Paul Rasmussen and David E. A. Catcheside

School of Biological Sciences, Flinders University, Adelaide, South Australia 5001, Australia

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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<sup>+</sup> progeny of a translocation heterozygote, in which the translocation breakpoint separates *cog* from the 5<sup>′</sup> 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.

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**M**EIOTIC 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; Schultz and Szostak 1990; Symington et al. 1991; Detloff et al. 1992; Malone et al. 1992), and other fungi (Gutz 1971; Stambberg 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<sup>+</sup>* 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<sup>−</sup>* (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-2<sup>+</sup>* 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 centromere-distal of the 3<sup>′</sup> end of the *his-3* coding sequence (Bowring and Catcheside 1991) within a region 2.3–3.2 kb from the 3<sup>′</sup> end of *his-3* (Yeadon and Catcheside 1995) and adjacent to the 5<sup>′</sup> end of the lyso<sub>phospholipase</sub> (*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<sup>−</sup>* is on the normal-sequence chromosome. Catcheside and Angel (1974) concluded that *cog<sup>−</sup>* 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...
translocation and normal-sequence chromosomes are paired. Catche- side (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 Catche- side 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 adenine, 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 HindIII/EcoRI 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 MgCl2, an annealing temperature of 52°C, 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), cog2 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 (Catche- side 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 XbaI, PvuII, MluI, NotI, and Sphi sites for insertion of exogenous DNA (Figure 1).

IgGκB, a human immunoglobulin kappa light chain gene, was cloned into XbaI/SphI of pDV2 and pDV3 (Figure 1), generating pDV2-B5 and pDV3-B5, respectively. pNK2 (Sachs et al. 1997) was digested with HindIII and EcoRI, giving a 2-kb fragment that includes TK inserted between the Neurospora arg-2 promoter and terminator sequences (Sachs et al. 1997). The arg-2-TK fragment, after end-filling with Klenow, was ligated into the Pmel 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, cog2, 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 hetero- karyon 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 (AhdI, PvuII, PvuI, and SphiI, Figure 1) and transformed (Bowring and Catche- side 1993) into spheroplasts (Case et al. 1979)

**Figure 1.—Vectors pDV2 and pDV3.** Each vector contains a 5’-truncated his-3 gene, cog2 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

<table>
<thead>
<tr>
<th>Stock no.</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2326</td>
<td>a; fl</td>
</tr>
<tr>
<td>T2327</td>
<td>A; fl</td>
</tr>
<tr>
<td>T4394</td>
<td>a, his-3 K504, cogL, ad-3; rec-2</td>
</tr>
<tr>
<td>T11249</td>
<td>arg-1; A; am, rec-2</td>
</tr>
<tr>
<td>T11250</td>
<td>arg-1; A; rec-2</td>
</tr>
<tr>
<td>T11255</td>
<td>arg-1; A; am, rec-2</td>
</tr>
<tr>
<td>T11257</td>
<td>arg-1; A; rec-2</td>
</tr>
<tr>
<td>T11264</td>
<td>A, his-3 K1201, cogL, ad-3; am, rec-2</td>
</tr>
<tr>
<td>T11281</td>
<td>a, his-3 K26, cogL; am, rec-2</td>
</tr>
<tr>
<td>T11439</td>
<td>arg-1, a, his-3 TM429, cogL; rec-2</td>
</tr>
<tr>
<td>T11440</td>
<td>arg-1, a, his-3 TM429, cogL; rec-2</td>
</tr>
<tr>
<td>T11442</td>
<td>arg-1, a, his-3 TM429, cogL; rec-2</td>
</tr>
<tr>
<td>T11630</td>
<td>a, his-3 K458, cogL; rec-2</td>
</tr>
<tr>
<td>T11631</td>
<td>a, his-3 K458, cogL; rec-2</td>
</tr>
<tr>
<td>T11644</td>
<td>A, his-3 K458, cogL; rec-2</td>
</tr>
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<td>T11668</td>
<td>a, lys-4, cogL; ad-3; am, rec-2</td>
</tr>
<tr>
<td>T11670</td>
<td>a, lys-4, cogL; ad-3; rec-2</td>
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<td>a, lys-4, his-3 K480, cogL; ad-3; am, rec-2</td>
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</tr>
<tr>
<td>T11690</td>
<td>A, his-3 K480, (arg2-TK), cogL; rec-2</td>
</tr>
<tr>
<td>T11704</td>
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</tr>
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</tr>
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<td>a, his-3 K26, (arg2-TK), cogL; rec-2</td>
</tr>
<tr>
<td>T11730</td>
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</tr>
<tr>
<td>T11738</td>
<td>A, his-3 K480, (IGcB), cogL; rec-2</td>
</tr>
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<td>a, his-3 K26, (IGcB), cogL; rec-2</td>
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<td>T11761</td>
<td>a, his-3 K480, cogL; am, rec-2</td>
</tr>
<tr>
<td>T11762</td>
<td>a, his-3 K480, cogL; am, rec-2</td>
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<tr>
<td>T11762</td>
<td>a, his-3 K480, cogL; am, rec-2</td>
</tr>
<tr>
<td>T11700</td>
<td>a, his-3 K480, cogL; am, rec-2</td>
</tr>
<tr>
<td>T11704</td>
<td>a, his-3 K480, cogL; am, rec-2</td>
</tr>
<tr>
<td>T11739</td>
<td>a, his-3 K480, cogL; am, rec-2</td>
</tr>
<tr>
<td>T11761</td>
<td>a, his-3 K480, cogL; am, rec-2</td>
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</tr>
<tr>
<td>T11762</td>
<td>a, his-3 K480, cogL; am, rec-2</td>
</tr>
</tbody>
</table>

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-I C102t.

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% sucrose, and supplements as required) and incubated for 24 hr. Colonies were picked into slopes (2% Vogels salts, 2% sucrose, and supplements as required) and incubated for 3 days. Since the strains with mosaic versions of cogL; rec-2 had frequency recombination phenotype of his-3, the parental origin (Catcheside et al. 1995), was PCR amplified and sequenced in each of the strains with mosaic versions of cogL; rec-2 (T11149 with pDV3-B5 and T11160 with pDV3-B5, respectively). PCR was used to identify presence of the IgG 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 T11644 with pDV3-B5 and T11630 with pDV2-B5, respectively. PCR was used to identify presence of the IgG 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 T11644 with pDV3-B5 and T11630 with pDV2-B5, 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 his-3 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. T11702 and T11204 (Table 1), generated by transfection of T11644 and T11630, respectively, with pDV-derived constructs each including an endoglucanase 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 cogL and cogG.
**Figure 3.**—The frequency of TK$^+$ amongst His$^+$ progeny of crosses heterozygous for TK. (A) Homozygous cog$^+$, K480, and TK in cis (T11704 × T11739 and T11704 × T12004). (B) Homozygous cog$^+$, K480, and TK in trans (T11738 × T11726, T12002 × T11726, T11761 × T11730, and T11760 × T11730). (C) Heterozygous cog$^+$ with K480, TK, and cog$^+$ in cis (T11704 × T11318). (D) Heterozygous cog$^+$ with K26, TK, and cog$^+$ in cis (T11681 × T11725). A dashed line indicates that a sequence other than TK (IG$^+$, egl3, or the native sequence) is present in the his-3–cog interval. The centromere is to the left side of the figure.

**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.

**TK heterozygotes**

<table>
<thead>
<tr>
<th>his-3</th>
<th>TK</th>
<th>cog (L or E)</th>
<th>TK$^+$</th>
<th>TK$^-$</th>
<th>% TK$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>63</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**TK$^+$ amongst His$^+$ progeny**

A

|       |    |             | 123    | 5      | 96.1     |

B

|       |    |             | 0      | 64     | 0        |

C

|       |    |             | 2      | 62     | 3.1      |

**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$^+$ 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-
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$^+$ and cog$^-$ 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$^-$ 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$^+$ 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$^+$, T11257 is cog$^-$ in phenotype.

Although the cog region sequences in T11249 and T11250 are mostly of cog$^+$ (Lindegren 25a) origin, the centromere-distal end of each sequence is from cog$^-$ (Emerson a; Figure 5). In contrast, T11257 has sequence from cog$^-$ 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$^-$; the underlined C is T and the T is A in cog$^+$. We can conclude that one or both of these bases is essential for the high-frequency recombination phenotype of cog$^+$. Of the other 11 sequence variations that distinguish cog$^+$ from cog$^-$ (Yeadon and Catcheside 1995), we can conclude that the central 6 (including a 9-base palindrome arising from an insertion of TGG in cog$^-$) 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$^+$) are required for the high-frequency recombination phenotype of cog$^+$. In addition, since cog$^+$ retains weak hotspot activity, even if the two nucleotides identified above are the only positions essential for the high-frequency recombination phenotype of cog$^+$, 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$^+$ × his-3 K26 IgGxB cog$^-$), one was TK$^+$, and of 32 His$^+$ progeny of T11704 × T12004 (his-3 K480 TK cog$^+$ × his-3 K26 egl3 cog$^+$), none was TK$^+$ (Fisher’s exact test: $P_{\text{ex}} = 0.38$). Of 32 His$^+$ progeny of T11738 × T11726 (his-3 K480 IgGxB cog$^+$ × his-3 K26 TK cog$^+$), 31 were TK$^+$, and of 32 His$^+$ progeny of T12002 × T11726 (his-3 K480 egl3 cog$^+$ × his-3 K26 TK cog$^+$), 30 were TK$^+$ (Fisher’s exact test: $P_{\text{ex}} = 0.50$). Of 32 His$^+$ progeny of T11761 × T11730 and 32 of T11762 × T11730 (his-3 K480 cog$^+$ × his-3 K26 TK cog$^+$), 32 and 30, respectively, were TK$^+$ (Fisher’s exact test: $P_{\text{ex}} = 0.25$). 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$^+$, 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$^-$. 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**

<table>
<thead>
<tr>
<th>Cross</th>
<th>his$^+$ ad$^+$</th>
<th>his-3 ad-3</th>
<th>his$^+$ ad-3</th>
<th>his-3 ad$^+$</th>
<th>Parental</th>
<th>Recombinant</th>
<th>rf (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T11249 × T4394</td>
<td>147</td>
<td>104</td>
<td>4</td>
<td>1</td>
<td>251</td>
<td>5</td>
<td>1.95</td>
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<td>T11250 × T4394</td>
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<td>118</td>
<td>2</td>
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<td>253</td>
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<td>1.17</td>
</tr>
<tr>
<td>T11257 × T4394</td>
<td>116</td>
<td>123</td>
<td>9</td>
<td>8</td>
<td>239</td>
<td>17</td>
<td>6.64</td>
</tr>
</tbody>
</table>

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).
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. Crossover type 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^4. 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 crossover type 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, Ascoholus 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^2 (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^2 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 Lichtten 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
Recombination by Template Switching

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.

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 coincident with the motif necessary for the cog’ 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’ 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’ 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 exchange cannot pass the breakpoint to give conversion of proximal his-3 alleles (Figure 7f 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 cog5, interallelic recombination occurs with a frequency of 11/105 viable spores whether rec-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-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 cog5, the presence of rec-2 reduces the frequency of His+ progeny of a cross to K504 from 167.0/105 to 5.1/105 viable spores (Catcheside and Angel 1974), suggesting that initiation at the proximal “hotspot” occurs 20- to 50-fold less frequently than it does at cog5. In contrast, in crosses of TM429 to another proximal allele (TM428) on a normal sequence chromosome that carries cog5, rec-2 reduces interallelic recombination ~20-fold (Catcheside and Angel 1974), suggesting the expected effect of the regulatory gene on recombination initiated at cog5, despite the presence of the translocation. Substitution of K874 for TM429 increases the frequency of His+ progeny <3-fold, from 18.8/105 (Catcheside and Angel 1974) to 52.7/105 viable spores (Angel et al. 1970), suggesting that the translocation has only a small effect on recombination compared to the effect of rec-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 cog5 stimulates events at this site. If this were the case, it is difficult to explain why this stimulation occurs only in cis to cog5 without concluding that the “stimulating effect” is due to a single recombination event, initiated at cog5 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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