The Effect of Heterologous Insertions on Gene Conversion in Mitotically Dividing Cells in Drosophila melanogaster

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\textbf{ABSTRACT}

We examined the influence that heterologous sequences of different sizes have on the frequency of double-strand-break repair by gene conversion in \textit{Drosophila melanogaster}. We induced a double-strand break on one X chromosome in female flies by Pelement excision. These flies contained heterologous insertions of various sizes located 238 bp from the break site \textit{in cis} or \textit{in trans} to the break, or both. We observed a significant decrease in double-strand-break repair with large heterologous insertions located either \textit{in cis} or \textit{in trans} to the break. Reestablishing the homology by including the same heterologous sequence \textit{in cis} and \textit{in trans} to the double-strand break restored the frequency of gene conversion to wild-type levels. In one instance, an allelic nonhomologous insertion completely abolished repair by homologous recombination. The results show that the repair of a double-strand break by gene conversion requires chromosome pairing in the local region of the double-strand break.

\textbf{DOUBLE-STRAND-break repair by gene conversion is a complex process that is ubiquitous in all organisms. This process maintains the integrity of eukaryotic genomes in somatic cells (Jasin 2000) and reinitiates DNA replication after the replication fork passes through a single-strand nick in the DNA (Kuzminov 1999). Furthermore, gene conversion is responsible for repairing the majority of DNA double-strand breaks that occur in the G\textsubscript{2} phase of the cell cycle (Takata \textit{et al.} 1998). Double-strand-break repair is also responsible for crossing over during meiosis and, as such, is a fundamental process in the formation of genetic variability in a species (Stahl 1996). Double-strand-break repair by gene conversion involves two distinct DNA sites. The first is the site at which the double-strand break occurs; the second is the site from which sequence information is copied. The DNA sequence flanking the break is processed by exonucleases leaving 3’ single-strand extended ends (at least in \textit{Saccharomyces cerevisiae}; Paques and Haber 1999). Thus, at least some of the information flanking the double-strand break is lost. DNA replication primed by the resected single-strand end regenerates the lost information (Haber 1995). The DNA replication template sequence therefore donates information to the broken chromosome. For this reason, we refer to the chromosome that has experienced the double-strand break as the recipient and the chromosome that provides sequence information as the donor.

The repair of DNA double-strand breaks by homologous recombination requires that the two interacting DNA molecules have very similar sequences. The amount of homology required for repair has been studied in two main contexts. One focuses on the fraction of sequence identity shared between the recipient and donor DNA sequences. The frequency of gene conversion is greatest when the two sequences are identical and declines as the percentage identity between the two sequences decreases. In \textit{Escherichia coli} and \textit{S. cerevisiae}, and presumably also in other organisms, two different systems evaluate the sequence identity between the interacting molecules (Kuzminov 1999; Paques and Haber 1999). During the search for homology the initial match made by the recA/RAD51 protein is promoted (at least \textit{in vitro}) between sequences with as much as 10% sequence difference (Bazemore \textit{et al.} 1997). The mismatch repair system then tests for sequence matching and rejects those that contain mismatched base pairs (Modrich and Lahue 1996; Evans and Alani 2000). The mismatch repair system is thus responsible for most of the sequence specificity during double-strand-break repair by gene conversion.

The homology requirements for double-strand-break repair have also been examined in the context of the extent of donor sequence homology. This is often expressed in terms of the MEPS, or minimal efficient processing segment. This is the smallest amount of contiguous identical sequence between the donor and recipient sites that is required to initiate efficient double-strand-break repair by gene conversion or recombination. The MEPS varies from \textasciitilde{}25–50 bp in \textit{E. coli} and bacterio-
phage T4 to \( \sim 200 \) bp in eukaryotic systems (Singer et al. 1982; Rubnitz and Subramani 1984; Shen and Huang 1986; Linkay et al. 1987; Thaler and Noordewier 1992; Jinks-Robertson et al. 1993). In these analyses various heterologous sequences were used as markers for the occurrence of gene conversion, but the size and sequence of the heterologous sequences were not systematically varied.

We report an experiment to test the effect of various heterologous insertions on gene conversion in a constant chromosomal context. In our experiments, the recipient chromosome carries the \( w^{whd80k17} \) allele, which has a \( P \)-element insertion in exon 6 of the \( white \) gene. Double-strand DNA breaks are induced in this mutant \( white \) gene by \( P \)-element excision, and repair of the break produces a functional \( white \) gene (Figure 1; Engels et al. 1990; Gloor et al. 1991). The donor site in these experiments is allelic to the recipient \( white \) gene and carries a heterologous sequence ranging in size from 25 to \( \sim 13,000 \) bp inserted 238 bp downstream of the break site. Chromosomes carrying one of these insertions were tested for their ability to act as donors for gene conversion as measured by reversion of the \( w^{whd} \) allele to wild type following \( P \)-element excision. We measured this effect in both the developing germ line and in somatic tissues.

**MATERIALS AND METHODS**

**Heterologous template production:** \( pP[\text{wall}] \) is a plasmid that contains a mini-\( white \) gene flanked by \( P \)-element ends (Nassif et al. 1994). Sequences cloned into the polylinker, which lies 238 bp downstream of the \( w^{whd} \) \( P \)-element insertion site, were delivered to the genome by \( P \)-element-mediated transformation (Spradling and Rubin 1982) and sequences were targeted to the \( white \) gene by \( P \)-element-mediated targeting (Nassif et al. 1994). The construction of templates that contained either 25, 242, or 7897-bp insertions or a 136-bp deletion has been described previously (Nassif et al. 1994). A template carrying the \( \sim 13,000 \)-bp forked gene has also been described (Keeler et al. 1996). The plasmid \( pP[\text{wall}-\lambda 8Kb] \) contains 8428 bp of bacteriophage \( \lambda \) DNA constructed by cloning a 8428-bp \( 	ext{BglI/ BclI restriction fragment into the } \text{BamHI site of } pP[\text{wall}] \). The plasmid \( pP[\text{wall}-\lambda 3Kb] \) contains 3320 bp of the bacteriophage \( \lambda \) sequence. It was made by digesting \( pP[\text{wall}-\lambda 8Kb] \) with \( 	ext{BamHI and XhoI}, \) using DNA polymerase to fill in the ends, followed by treatment with DNA ligase to join the resulting blunt ends. An EcoRI fragment from the human Huntington’s gene (Huntington’s Disease Collaborative Research Group 1993) was cloned into the EcoRI site of the \( pP[\text{wall}] \) polylinker to make a template containing 4168 bp of human genomic sequence.

**Genetic techniques:** Flies were reared on cornmeal-sugar-agar medium at room temperature (25°) unless noted. Genetic symbols not described here are found in Lindsley and Zimm (1992) or in Flybase at http://flybase.bio.indiana.edu/.

**Drosophila strains:** The \( w^{whd80k17} \) allele (hereafter \( w^{whd} \)), which contains a single \( P \)-element insertion in exon 6 of the \( white \) locus, causes a bleach-white eye phenotype (O’Hare et al. 1984; Engels et al. 1990). Several chromosomes containing \( y^{+}, y, y^{c}, ac, \) or \( y \) \( ac \) or \( y \) on the telomeric side of the \( w^{whd} \) allele and \( spl \) or \( spl^{*} \) on the centromeric side of the \( w^{whd} \) allele were constructed by meiotic recombination.

**Statistical methods:** \( P \)-element excision frequently occurs in developing germ cells before meiosis (Daniels and Chovnick 1993). Since the double-strand breaks are induced in our experiments by \( P \)-element excision, this often results in the recovery of clustered double-strand-break repair events in the progeny of a single fly. Engels (1979) showed that for a given experiment the most accurate estimate of the frequency of repair when clustering was pronounced was the unweighted average, while the most accurate estimate of unclustered events was the weighted average. The appropriate method could be identified by calculating the variance of the unweighted or weighted average and choosing the one with the smallest variance. This method was applied to all our measurements, with the result that some estimates of the frequency of repair were weighted averages and some are unweighted averages. Generally, we found that the weighted average was most appropriate when the reversion frequency was \( >5\% \) and the unweighted average was most appropriate when the reversion frequency was \( <5\% \). Clustering also has the effect of causing large variations in the reversion frequency because a large cluster derived from a single family could dramatically alter the reversion frequency. Therefore, critical experiments were conducted in duplicate or triplicate to show that the results were reproducible.

**Quantitation of somatic reversion:** A Nikon Coolpix 880 digital camera was used to take pictures of each eye of the Drosophila females in which double-strand-break repair was induced by excision of the \( w^{whd} \) \( P \)-element. Images were processed and analyzed using Adobe Photoshop 5.5 as follows. The color balance of the image was adjusted automatically using the default settings. The selection wand was used to select all red tissue in the eye with tolerances of 20 and 40. Red tissue was selected until selection of red patches exclusive of other colors was no longer possible. The selected area(s) in the eye were then converted to black by making a new adjustment layer with a threshold value of 128 to ensure that a consistent shade of red was chosen for the edge of each spot. The free-form selection tool was used to trace the outline of each eye. Examination of the histogram of the enclosed area provided a measure of the total number of pixels in the eye and the percentage of the eye covered by black pixels. This percentage was noted for graphing and statistical analysis.
Results

We were interested in the effect of closely linked heterologous insertions in double-strand-break repair by gene conversion in the Drosophila genome. We placed insertions of heterologous sequence in cis or in trans to the location of a double-strand break made by P-element excision at the white locus in the developing germ line or in the soma.

Gene conversion in the germ line: The white gene in these experiments was flanked by one or more visible mutations in the yellow, acheta, or seute genes on the telomeric side and by the Notch\textsuperscript{white} allele on the centromeric side. These markers define a small genetic interval of <3 cM that contains the white locus. It was unlikely that the white allele would be separated from this interval by double meiotic recombination.

In this system precise repair of the double-strand break by gene conversion results in a reconstituted white gene on the recipient chromosome. Progeny that inherit such a chromosome have wild-type eye color (Gloor et al. 1991; Nassif and Engels 1993; Nassif et al. 1994). Therefore, the proportion of nonrecombinant progeny that have a wild-type eye color is a direct measure of the frequency of gene conversion in the developing germ line. Figure 1 shows the mating scheme used to measure the effect of heterologous insertions on the frequency of double-strand-break repair in the developing germ line. We counted nonrecombinant revertant and nonrevertant progeny from females that had insertions of different sizes on the donor chromosome.

The donor chromosomes in this study were constructed by P-element-dependent gene conversion from an ectopic donor derived from P\text{[wall]} (Nassif et al. 1994). As a result they contained single base differences with the recipient chromosome. We chose donor chromosomes that had conversion tracts extending from nucleotide positions -1025 to +238 or from sites -393 to +311 (all nucleotide sites are with reference to the white P-element insertion; nucleotide positions shown in Nassif et al. 1994). Conversion tracts of this length have between 8 and 11 nucleotide differences with the white allele, and previous work has shown that between 9 and 11% of white excisions should revert to wild type in the presence of these donors (Nassif and Engels 1993).

All the insertions on the donor chromosome were 238 bp downstream of the position of the white P-element insertion site on the recipient chromosome. We also used a chromosome that had a 7987-bp yellow gene insertion (Geyer and Corces 1987) at the same location, but on the recipient chromosome (N. Nassif and W. R. Engels, personal communication). This chromosome also contained seven single base differences with the normal white chromosome, located at nucleotide positions +1 to +884 with reference to the white P-element insertion site. Therefore, when the white\textsuperscript{c} chromosome was the recipient, there were 6–10 single base differences with the donor chromosomes. Figure 2 shows the sizes of the heterologous insertions in the seven donor white genes. Finally, one of the donor chromosomes carried a 136-bp deletion in the same position (Nassif et al. 1994).

The seven donor white genes with insertions of heterologous sequence ranging in size from 25 to ∼13,000 bp were tested for their ability to revert to the white allele following excision of the P-element. Our first test used a donor white gene that carried a 25-bp insertion. In two separate trials this donor yielded reversion frequencies of 7.9 and 10.9% (Table 1, line A), which are comparable to those observed by Nassif and Engels (1993) for similar donor sequences. Thus, the 25-bp insertion in the donor DNA sequence does not affect the reversion frequency significantly.

We next tested the effect of larger insertions on the reversion frequency of the white allele. We observed that insertions between 242 and 4168 bp caused an approximately threefold reduction in the reversion frequency (Table 1, lines C–E). Three separate trials with an insertion of the 7978-bp yellow gene, and one with an 8428-bp fragment of bacteriophage \(\lambda\), showed an approximately fivefold reduction in the reversion frequencies (Table 1, lines F and G).

The presence of a small deletion on the donor chromosome caused the reversion frequency to decrease by
Figure 2.—Configuration of the donor and recipient chromosomes. The recipient chromosome carries a \( P \)-element insertion in exon 6 of the white gene that results in a bleach-white eye phenotype. Precise loss of the \( P \)-element reverts this mutation to wild type. The donor chromosomes contain reverted white genes that have insertions of various sizes located 238 bp downstream of the site of the \( w^{hd} \) \( P \)-element insertion on the recipient. One donor chromosome has a deletion of 136 bp and has been described elsewhere (Nassif et al. 1994).

The reversion frequency decreased as the size of the heterologous insertion increased. Figure 3 shows a log-log plot of the reversion frequency vs. insertion size. The reversion frequency and insertion length showed a linear correlation \((r = -0.932, P < 0.001)\). We conclude that the insertions reduce the reversion frequency in a size-dependent manner and that this effect is independent of the particular insertion sequence. Furthermore, a similar reduction in reversion frequency occurs when the insertion is in cis to the double-strand break or in trans. Finally, we measured reversion with the \( w^{dy+} \) recipient chromosome opposite a donor chromosome containing either the 8428- or 3320-bp bacteriophage \( \lambda \) insertion or the \(~13,000\)-bp forked gene insertion. This experiment was done to see if there were any additional effect on the reversion frequency with different insertions in cis and in trans to the double-strand break. The combination of the small \( \lambda \) insertion and the yellow gene, or of the forked gene and the yellow gene, reverted at frequencies similar to those seen in the single insertion experiments (Table 1, lines L and N). Two separate trials with the combination of the forked gene in trans to the double-strand break and the Drosophila yellow

\( \sim 50\% \) (Table 1, line B). This configuration is equivalent to an insertion in the recipient chromosome. We therefore investigated the effect of placing a heterologous insertion in cis to the double-strand break. For this experiment we obtained a \( w^{dy+} \) recombinant chromosome containing the 7978-bp yellow gene in cis to the \( w^{hd} \) \( P \)-element. Female flies that carried the \( w^{dy+} \) recipient chromosome and a donor chromosome carrying the 25-bp insertion were tested for the production of revertant progeny. A comparison of lines F and H in Table 1 shows that the reversion frequency was similar when the 7978-bp yellow gene was in cis or in trans to the double-strand break. An important control for this experiment was to make the yellow gene insertion homozygous. This configuration restores the homology between the interacting chromosomes; therefore, we would expect the reversion frequency to be similar to that observed in the absence of an insertion. Reestablishment of the homology between the donor and recipient chromosomes in the region of the double-strand break indeed restored the reversion frequency to that observed in the absence of an insertion (Table 1, line I).

The reversion frequency decreased as the size of the
gene in cis resulted in reversion frequencies of 1.8 and 2.9% reversion (Table 1, line N). The results show that there is no effect on the gene conversion frequency when heterologous insertions are both in cis and in trans to the double-strand break.

Interestingly, two separate trials of the combination of the Drosophila yellow gene in cis and the large bacteriophage λ insertion in trans failed to yield a single revertant (Table 1, line M). We examined the X chromosomes prepared from salivary glands of larvae heterozygous for the w^{bly+} chromosome and the 8428-bp bacteriophage λ insertion and found that their appearance was normal. Therefore, the abnormally low reversion frequency was not caused by a gross chromosome rearrangement. The low reversion frequency may be specific to the combination of the w^{bly+} and λ sequences because the heterozygous combination of the other two insertions in trans to the w^{bly+} chromosome yielded revertants.

**Gene conversion in somatic cells:** We next examined if a heterologous sequence affected the repair of double-strand breaks by gene conversion in somatic cells. We adapted the mating scheme shown in Figure 1 by employing a w^{118} donor allele in place of the revertant chromosome. The w^{118} allele has a bleach-white eye phenotype because of a deletion of sequences in the white gene that include the first exon (Kurkulos et al. 1991), but is wild type for the exonic sequence around the w^{bd} P element. The recipient in these experiments was either the w^{bd} or the w^{bly+} chromosome. Gene conversion occurring during eye development would cause eye color mosaicism. The proportion of the eye covered by red tissue was extremely variable because the gene conversion event that led to the restoration of the recipient w^{bd} white gene could occur at any time during development. For example, a large red patch would result if gene conversion occurred early in development and a small red patch would be seen if gene conversion occurred late in development. Images of individual eyes from the female in which the w^{bd} P element was excising were captured with a digital camera and the proportion of red tissue in each eye was determined as described in the MATERIALS AND METHODS. The proportion of red tissue represents a measure of gene conversion occurring in the developing eye tissue.

When the w^{bd} P element was excised in the absence of a flanking heterologous sequence, we observed an average of 26.1 ± a standard deviation of 12.1% ($n = 323$) of the eye being covered by red pigment. In contrast, excision of the P element on the w^{bly+} chromosome resulted in 9.8 ± 8.7% ($n = 331$) and 9.5 ± 7.9% ($n = 261$) of the eye being covered by red pigment.

### TABLE 1
Reversion frequencies for templates with allelic insertions

<table>
<thead>
<tr>
<th>Origin</th>
<th>Size (bp)</th>
<th>Location relative to DSB</th>
<th>Reverted chromosomes/total chromosomes scored</th>
<th>Reversion frequency (% ± SD) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Synthetic</td>
<td>25</td>
<td>trans</td>
<td>35/417</td>
<td>7.9 ± 1.3^a</td>
</tr>
<tr>
<td>B Deletion</td>
<td>−136</td>
<td>trans</td>
<td>51/785</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td>C Yeast</td>
<td>242</td>
<td>trans</td>
<td>61/1168</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>D λ</td>
<td>3320</td>
<td>trans</td>
<td>92/2155</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>E Human</td>
<td>4168</td>
<td>trans</td>
<td>101/4104</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>F Drosophila</td>
<td>7897</td>
<td>trans</td>
<td>61/3956</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>G λ</td>
<td>8428</td>
<td>trans</td>
<td>92/3370</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>H Drosophila</td>
<td>7897/25</td>
<td>cis/trans</td>
<td>54/2312</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>I Drosophila</td>
<td>7897/7897</td>
<td>cis/trans</td>
<td>198/2941</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>J Drosophila/yeast</td>
<td>7978/242</td>
<td>cis/trans</td>
<td>44/2429</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>K Drosophila/deletion</td>
<td>7978/−136</td>
<td>cis/trans</td>
<td>7/1516</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>L Drosophila/λ</td>
<td>7978/3320</td>
<td>cis/trans</td>
<td>34/2786</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>M Drosophila/λ</td>
<td>7987/8428</td>
<td>cis/trans</td>
<td>0/3442</td>
<td>0</td>
</tr>
<tr>
<td>N Drosophila</td>
<td>7978/13000</td>
<td>cis/trans</td>
<td>24/1314</td>
<td>1.8 ± 0.4</td>
</tr>
</tbody>
</table>

* Statistical methods are explained in MATERIALS AND METHODS.
* Recombinant chromosomes on the centromeric side of the white locus were not counted separately in this experiment. Therefore this calculation assumes a 1.5% recombination frequency between white and split.
We examined how local pairing between homologs affected double-strand-break repair by gene conversion. Nassif and Engels (1993) had previously shown an inverse relationship between the reversion frequency and the number of single base differences between the donor and recipient chromosomes. In our experiments the number of single base differences between the donor and recipient chromosomes was similar, being held between 6 and 11 in each case. We found that heterologous sequences located near the double-strand break dramatically decreased the ability of the allelic homologous sequence to be used as a donor for gene conversion. This effect depended on the size of the heterologous sequence—larger sequences resulted in significantly less gene conversion than smaller sequences. We observed the effect in both the developing germ line and in somatic tissues, suggesting that the constraints on what constitutes a suitable donor sequence are similar in both tissues.

DNA double-strand-break repair by gene conversion requires that a DNA sequence homologous to that flanking the site of the double-strand break be identified and copied. The biochemical steps underlying this homology search are relatively well defined. The ubiquitous recA/RAD51 family of proteins mediate the homology search (Haber 2000), and the quality of the pairing between the donor and recipient DNA molecules is assessed by the mismatch repair system (Modrich and Lahue 1996; Evans and Alani 2000). However, other factors that influence the homology search in vivo are poorly characterized. One important question is, under what situations are homologous sequences recognized as suitable donors for double-strand-break repair by gene conversion?

One important determinant for efficient gene conversion of a heterologous sequence is the extent of flanking sequence homology. This has been determined in many systems. The MEPS, which represents the smallest unit of flanking homology that allows for efficient gene conversion, ranges from ~25 bp in the E. coli recBCD pathway to ~200 bp in S. cerevisiae and other eukaryotes (Singer et al. 1982; Rubnitz and Subramani 1984; Shen and Huang 1986; Liskay et al. 1987; Thaler and Noorde-Dewier 1992; Jinks-Robertson et al. 1993; Dray and Gloor 1997). In experiments of this type, a heterologous insertion is flanked by different lengths of sequence homologous to recipient sequence. The frequency of gene conversion increases with increasing amounts of flanking homology in these experiments. In our experiments, the amount of flanking sequence homology was constant but the size of the heterologous sequence varied. Thus, each of the insertions was expected to disrupt the MEPS in a similar way and thus was expected to cause a similar reduction in gene conversion. Our experiments showed that this was not the case and that the size of the insertion itself had a dramatic effect on gene conversion.

A second important determinant is the location of the interacting DNA sequences. Homologous sequences promote different frequencies of gene conversion in a position-dependent fashion in many systems. For example, during double-strand-break repair by gene conversion in mitotically dividing Drosophila cells in the germ line, homologous sequences are used as gene conversion donors with the following efficiency: sister chromatid > allelic site on homologous chromosome > in cis
to the double-strand break on the same chromosome > ectopic sequence located elsewhere in the genome (Engels et al. 1990; Gloo et al. 1991; Johnson-Schlitz and Engels 1993; Nassif and Engels 1993; Engels et al. 1994; Nassif et al. 1994). Similarly, homologous sequences are used for gene conversion with similar position-dependent efficiencies in mitotically dividing S. cerevisiae cells and in cultured mammalian cells (Lichten and Haber 1989; Baker et al. 1996; Burgess and Kleckner 1999). In our experiments the donor and recipient sites were always allelic, and the site of the heterologous insertion was held constant. Therefore, the location of the interacting sites was identical in every instance.

It is intriguing to note that site-specific recombination between two target sites also occurs more frequently in cis than in trans in both Drosophila and S. cerevisiae (Golic and Golic 1996a; Burgess and Kleckner 1999). In these experiments the recombination rate varied inversely with the distance between the two target sites. The rate of site-specific recombination varies directly with the local concentration of the interacting sites, and therefore the site-specific recombination rate can be used to measure the effective distance between these sites. However, Engels et al. (1994) found that the rate of gene conversion in cis was unaffected by distance. This suggests that random collision between the break site and the donor site contributes to, but is not the major factor controlling, the homology search. Furthermore, it indicates that site-specific recombination and homologous recombination have different means of identifying homologous sequences.

Chromosome pairing is a third factor that determines whether a homologous sequence is recognized as a potential donor molecule during gene conversion. It is now well established that homologs are paired in mitotically dividing eukaryotic cells (Fung et al. 1998; Burgess et al. 1999) and that this pairing is involved in a broad array of genetic phenomena (Wu and Morris 1999). However, the relationship between gene conversion (or homologous or site-specific recombination) and the pairing of homologous chromosomes in mitotically dividing cells is characterized poorly. One study in yeast suggested that broken chromosome ends were promiscuous in that two broken ends could locate each other anywhere in the genome if they shared sufficient sequence homology (Haber and Leung 1996). Engels et al. (1990) found that an allelic homologous sequence was used as a donor for gene conversion about fourfold less often when it was located in an inversion loop than when it was not. This was surprising because in this instance the homologous sequence was located in the middle of the inversion loop and therefore had several megabases of flanking sequence homology. Golic and Golic (1996b) examined the effect of mitotic chromosome pairing on site-specific recombination initiated by FLP recombinase. They found that the introduction of a heterozygous inversion loop between the two interacting FLP-recombinase target sites resulted in a reduction in recombination in every instance. Increasing the length of the cell cycle suppressed the effect of the inversion loop on site-specific recombination. The interpretation was that somatic pairing of homologs requires more time when an inversion loop is present. This inter-
Reversion Frequency

A.  

\[ \text{P-wd}^{hd} \]

25 bp  

7.9-10.9%

B.  

6.7-11.7%

C.  

1.1-1.8%

D.  

1.9-2.3%

E.  

0-2.9%

F.  

13.6%

Figure 5.—Reversion frequencies of paired and unpaired chromosomes. The reversion frequencies of the large insertions (>7000 bp) given in Table 1 are shown beside the hypothetical chromosome pairing in the region of the double-strand break. The \( w^{hd} \) element is diagrammed as the line with two divergent arrowheads, the white gene is shown as a thin black line, and the heterologous insertions are shown as solid or as open boxes. The sizes of the heterologous insertions and the white gene sequences are not shown to scale. The heterologous insertions in parts A–E are 238 bp downstream of the \( w^{hd} \) element insertion site; the insertion in F is 2025 bp upstream of the same site.

Interpretation correlates with the recent observation that homologous pairing in Drosophila somatic cells proceeds by random collision of pairing sites (Fung et al. 1998); therefore, a longer cell cycle would allow greater pairing between homologs even if there were a heterozygous inversion.

We suggest that the insertion of heterologous sequences in the donor chromosomes disrupts chromosome pairing and that this disruption causes the observed reduction in gene conversion. In our experiments it is likely that the heterologous insertions disrupt chromosome pairing only in the immediate region of the insertion. Figure 5 summarizes the data for the large heterologous insertions and outlines a model for how this disruption could occur. Chromosomes that are well paired in the local region of the double-strand break, as shown in Figure 5, A and B, yield gene conversion frequencies >7%. These frequencies are similar to those observed by Nassif and Engels (1993) when the two allelic sites differed only by single base substitutions. In contrast, chromosomes that are not well paired in the local region of the double-strand break (Figure 5, C–E) show significantly lower frequencies of gene conversion. In these configurations typically <2% of the double-strand breaks are repaired by gene conversion.

A similar chromosomal configuration is shown in Figure 5F. Here, a copia transposable element is inserted 2025 bp upstream and in trans to the insertion site of the \( w^{hd} \) element. In this instance 13.6% of the double-strand breaks are repaired by gene conversion (Engels et al. 1990). As discussed above, the MEPS for eukaryotic systems is at least 200 bp. Therefore, the heterologous insertions in the configurations shown in Figure 5, C–E, are located at approximately one MEPS from the dou-
ble-strand break, while the insertion in Figure 5F is much farther away. The insertion in Figure 5F would therefore not be expected to have an effect on the gene conversion frequency. This may account for the difference in the frequency of gene conversion in these different situations.

Double-strand-break repair is thought to initiate meiotic recombination in eukaryotes, including Drosophila (Stahl, 1996; McKim and Hayashi-Hagihara, 1998; Keeney et al., 1999; Romanienko and Camerini-Otero, 1999). It is interesting to note that the frequency of meiotic recombination at the rosy locus in Drosophila is not affected by the presence of heterologous insertions or base-pair polymorphisms (Hilliker et al., 1994). Nassif and Engels (1993) have previously shown that single base differences between the donor and recipient molecules reduce gene conversion in mitotically dividing cells. Here, we show that double-strand-break repair is reduced when either the donor or recipient contain an insertion. Thus, these experiments further support the idea that double-strand-break repair in mitotically dividing cells of Drosophila has different homology requirements than double-strand-break repair during meiotic recombination.

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